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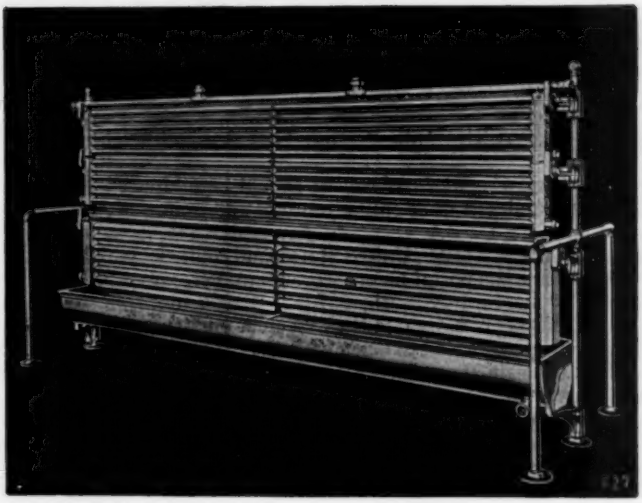

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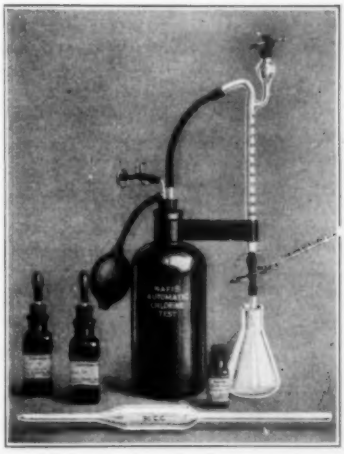

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The report further states that the sources of dairy farm water are more likely to be polluted with fecal bacteria than those of other farms, because of the extensive distribution of cow manure.

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THE EFFECT OF LECITHIN IN DAIRY PRODUCTS UPON BUTTER FAT DETERMINATIONS*

O. W. CHAPMAN

Section of Dairy Industries, Iowa State College, Ames, Iowa

INTRODUCTION

The loss of butter fat in dairy manufacturing processes, such as churning, was considered to be relatively unimportant when tests for the fat content were made by the regular Babcock test. But since the introduction of the butyl alcohol modification of the Babcock test, and the wider use of ether extraction methods, the fat losses appear to be considerably greater than was formerly supposed. Tested by the older method, the fat content of buttermilk showed tests of about 0.2 per cent. Tests recently made on a large number of samples from various creameries have shown that the average fat content of the buttermilk from Iowa creameries is 0.7 per cent. The question now arises, which method, if either, gives the true percentage of fat?

LITERATURE REVIEW

Thurston (12) has indicated that fat is not the only constituent of milk which is indicated by the fat tests, but that the results are influenced by the presence of lecithin. This appears to be quite probable, because of the similarity of lecithin to fat in its properties, such as its solubility. Whether or not the lecithin occurs in large enough amounts to cause an appreciable error in fat tests can be determined by comparing the amounts of lecithin and of fat in various dairy products.

That milk contains no lecithin is the belief of Schlossman (10), who reports that the phosphorus compounds which are generally believed to be lecithin, are in reality decomposition products of casein. This view is not held by other investigators, who have shown that lecithin is present in milk, and have determined the

* Received for publication June 9, 1928.

amount which is present. The results of some of these determinations made on cow's milk are given in table 1.

These results indicate that the average amount of lecithin present in milk is about 0.075 per cent. If we consider milk as having an average fat content of 3.6 per cent, then the error in the fat determination due to the presence of lecithin would be about two per cent, provided that the entire amount of lecithin figured in the determination.

While most of the results reported are for milk, some determinations have been made on the amount of lecithin in other products. Bordas and de Raczkowski (2) found 0.334 per cent in cream containing 50.88 per cent fatty material. Dornic and Daire (5) report values of 0.0905 and 0.0944 per cent for raw

TABLE 1

Lecithin content of cow's milk as found by various investigators

AUTHOR	PER CENT LECITHIN	AVERAGE VALUES
Stoklassa (11)	0.09 –0.113	0.1015
Burow (3)	0.049 –0.1058	0.0535
Koch and Woods (7)	0.072 –0.086	0.0797
Nerking and Haensel (9)	0.0364–0.1163	0.0629
Glikin (6)	0.0158–0.1173	0.0765

cream and of 0.0651 for pasteurized cream. While it is evident from these results that the lecithin content of cream is higher than that of milk, the percentage error in the fat determinations due to the presence of lecithin is of course considerably less because of the high fat content of the cream. Cusick (4) found the lecithin content of butter to vary between 0.0433 and 0.0723 per cent. Since the fat content of butter is ordinarily greater than 80 per cent, the lecithin would exert practically no influence on the determination.

Because skimmed milk and buttermilk contain much less fat than milk or cream, small amounts of lecithin in these substances will cause a greater error in the fat determinations than in milk, cream or butter. But few determinations of lecithin have been reported on skimmed milk and buttermilk. Of these, Dornic

and Daire (5) found 0.0332 per cent of lecithin in buttermilk which contained 0.42 per cent of fatty material. Bischoff (1) found that the phosphatides remain for the most part in the buttermilk. Bordas and de Raczkowski (2) report 0.018 per cent in skimmed milk which contained 0.09 per cent of fatty material. The error due to lecithin in the fat determination would thus be about 8 per cent for buttermilk, and 20 per cent for skimmed milk.

The results obtained by these investigators indicate that the presence of lecithin in substances of low fat content will exert considerable influence upon the results of the determination of the fat content.

TABLE 2

Lecithin in milk products

MATERIAL TESTED	PER CENT OF FATTY MATTER	PER CENT OF LECITHIN			PER CENT OF THE EXTRACT THAT IS LECITHIN
	Average	Low	High	Average	
Milk	3.848	0.0345	0.0709	0.0447	1.6
Cream	45.70	0.1824	0.2155	0.1981	0.43
Skimmed milk	0.153	0.0082	0.0290	0.0165	10.78
Buttermilk	0.643	0.1036	0.1480	0.1302	20.25

EXPERIMENTAL WORK

The amount of lecithin in dairy products

The experimental work has consisted of two parts: First, the determination of the amount of phosphatides, calculated as distearyl lecithin, present in the ether extract of various dairy products, the extraction having been made by the Mojonnier modification of Röse-Gottlieb method. Second, the preparation of lecithin, and its addition to buttermilk in varying amounts, followed by the determination of fatty matter.

To determine lecithin, samples of milk or cream or other material were extracted in the regular manner. A number of these extracts were combined to give a sufficiently large sample. The ethers were then evaporated, the residue dried and weighed,

then taken up with anhydrous ether or chloroform, transferred to a platinum dish, evaporated to dryness, and then fused with K_2CO_3 and $NaNO_3$. The phosphorus was then precipitated with ammonium molybdate, then as $MgNH_4PO_4$ and finally converted to $Mg_2P_2O_7$. From this lecithin may be calculated by using the factor 7.27, considering the lecithin to be of the di-stearyl type. The results thus obtained represent the amount of lecithin in the ether extract, but not necessarily the total amount of lecithin present in the original material.

A summary of results is given in table 2.

The results show that the lecithin is extracted in amounts great enough to cause a considerable error in the fat determination of skimmed milk and buttermilk. For skimmed milk this error would be 10.78 per cent, and for butter milk, 20.25 per cent.

The effect of added lecithin on fat determinations

Lecithin was prepared from the yolks of eggs according to the method of MacLean (8). The results of the analysis of this material as prepared were, phosphorous 4.03 per cent and nitrogen 1.84 per cent. The values given by MacLean are, phosphorous 4.0 per cent and nitrogen 1.8 per cent.

Weighed amounts of lecithin were then added to buttermilk, and fat determinations made on the buttermilk alone, and on the buttermilk after the addition of the lecithin. Three methods of testing were used: (*a*) Babcock, using skim milk test bottles, (*b*) Butyl alcohol modification of the Babcock, also using skim milk bottles, and (*c*) the Mojonnier test. The difference in the tests divided by the amount of lecithin added to 100 cc. of butter milk and multiplied by 100 gives the percentage recovery of lecithin.

The results of these tests are summarized in tables 3, 4 and 5. The data show that the results of the fat tests are increased by the presence of lecithin, although all of the lecithin is not recovered by the tests. In the Babcock test, 71 per cent of the lecithin added affects the result, in the butyl alcohol modification, 68.5 per cent, and in the Mojonnier test, 76.5 per cent. The last result is lower than expected, in view of Glikin's (6) work.

TABLE 3

The effect of added lecithin on fat tests made by the Babcock method

WEIGHT OF ADDED LECITHIN IN 100 CC.	TEST BEFORE ADDITION OF LECITHIN	TEST AFTER ADDITION OF LECITHIN	DIFFERENCE	PER CENT OF ADDED LECITHIN WHICH IS RECOVERED
grams	*per cent*	*per cent*	*per cent*	
0.1046	0.075	0.09	0.015	14.3
0.1484	0.25	0.33	0.08	53.9
0.0540	0.105	0.19	0.085	157.4
0.1808	0.13	0.19	0.06	33.2
0.1035	0.11	0.22	0.11	106.3
0.1063	0.11	0.23	0.12	113.0
0.1044	0.10	0.175	0.075	71.8
0.2083	0.10	0.20	0.10	48.0
0.1075	0.10	0.15	0.05	46.5
0.2005	0.10	0.23	0.13	64.8
0.1526	0.09	0.20	0.11	72.1
Average				71.0

TABLE 4

The effect of added lecithin on fat tests made by the butyl alcohol method

WEIGHT OF ADDED LECITHIN IN 100 CC.	TEST BEFORE ADDITION OF LECITHIN	TEST AFTER ADDITION OF LECITHIN	DIFFERENCE	PER CENT OF ADDED LECITHIN WHICH IS RECOVERED
grams	*per cent*	*per cent*	*per cent*	
0.1088	0.47	0.578	0.108	99.2
0.1046	0.46	0.525	0.065	62.1
0.1484	0.65	0.78	0.13	87.6
0.2122	0.55	0.65	0.10	47.1
0.0540	0.37	0.41	0.04	74.0
0.0618	0.42	0.43	0.01	16.2
0.1808	0.60	0.75	0.15	83.0
0.1035	0.56	0.59	0.03	29.0
0.1044	0.47	0.535	0.065	62.3
0.2083	0.47	0.69	0.22	105.6
0.1075	0.46	0.50	0.04	37.2
0.2005	0.46	0.65	0.19	94.7
0.1510	0.48	0.61	0.13	86.1
0.2510	0.48	0.67	0.19	75.7
0.1526	0.48	0.58	0.10	65.5
0.2550	0.48	0.66	0.18	70.6
Average				68.5

It is evident then that the results of the determinations of the amount of lecithin present in milk products, as determined on the ether extracts, are too low, since only 76.5 per cent of the lecithin is recovered. When this correction is made, the average lecithin content of butter milk becomes 0.1702 per cent, rather than 0.1302 per cent. The 0.1302 per cent, however, represents

TABLE 5

The effect of added lecithin on fat tests made by the Mojonnier method

WEIGHT OF ADDED LECITHIN IN 100 CC.	TEST BEFORE ADDITION OF LECITHIN	TEST AFTER ADDITION OF LECITHIN	DIFFERENCE	PER CENT OF ADDED LECITHIN WHICH IS RECOVERED
grams	*per cent*	*per cent*	*per cent*	
0.1484	0.683	0.821	0.138	93.0
0.2122	0.580	0.762	0.182	85.7
0.0540	0.482	0.517	0.035	64.8
0.0618	0.488	0.543	0.055	89.0
0.1808	0.613	0.726	0.113	62.5
0.1000	0.612	0.694	0.082	82.0
0.1035	0.611	0.662	0.051	49.3
0.1063	0.611	0.666	0.055	51.7
0.1044	0.551	0.643	0.092	88.1
0.2083	0.551	0.729	0.178	85.5
0.1075	0.551	0.637	0.086	80.0
0.2005	0.551	0.723	0.172	85.7
0.1510	0.559	0.654	0.095	62.8
0.2510	0.559	0.752	0.193	76.9
0.1526	0.559	0.684	0.125	81.9
0.2550	0.559	0.775	0.216	84.7
Average.....				76.5

the amount which is removed with the fat. Then the average fat content of the buttermilk from Iowa creameries is 0.57 per cent, rather than 0.7 per cent.

SUMMARY AND CONCLUSIONS

Previous investigators have shown that milk and milk products contain phosphatides, and suggested that these substances might influence the results of fat determinations.

The work reported in this paper shows that skimmed milk

and buttermilk contain phosphatides in amounts large enough to give results in the determination of fat which are appreciably high. It also shows that lecithin, the principal phosphatide present in milk, when added to buttermilk, causes a high result. The increase is approximately the same for the three methods of testing which were used.

The work also indicates that the average fat loss in buttermilk is 0.57 per cent, rather than 0.7 per cent, the difference being due to the amount of lecithin which appears in the fat test.

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THE EFFECT OF DISTILLED WATER UPON THE TENDENCY TO COLONY FORMATION UPON PETRI PLATES*

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The macroscopic colony count has been used for many years as a measure of the bacterial population of milk. In the numerous laboratories throughout the country it is considered the standard method, supported as it is, by the American Public Health Association. The first edition of Standard Methods mentioned only the plate method, which seemed to be adequate for the kinds of milk produced at that time. The numbers of bacteria found in milk were high as a rule, and one had but to separate the bad from the worse. In the four subsequent editions of Standard Methods other methods have claimed attention and obtained supporters, somewhat to the detriment of the plate method. Paralleling this diversity of methods there likewise has been an improvement in the milk itself, for there is no longer the large spread between milk of high and low quality. Such conditions place a greater tax upon the method which was devised primarily to eliminate the poorest milk.

There is small wonder then that the plate method has seemed to be less accurate. Too much is expected of it today. It is not an instrument of precision, which fact is recognized by the revising committees, as is evidenced by the statement in the introduction to the various editions of Standard Methods. Furthermore, it has recently been shown that Standard Methods have not had a fair chance. The Report of the Referee for the Bacteriological Examination of Milk (1) indicates that many brands of peptone and beef extract are used in amounts varying as much as 100 per cent from those considered standard. The reaction of the medium, the time and temperature of incubation were frequently quite different from those permitted. Now the methods have

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been subjected to repeated criticism, which seem on thoughtful consideration, to be unwarranted. Thus, one would be inclined to think that much of this criticism would tend to disappear were Standard Methods adhered to a little more carefully. It is the purpose of this paper to show that this is true and that one factor has been overlooked in the previous discussions. Most of the criticism has been that there was little constancy in the method; that duplicate plates poured at the same time would vary to a great degree. The work of Wright and Thornton (2) tended to show that this criticism was justifiable because they carefully made large numbers of plates from the same dilution, taking every precaution that all variables were controlled. They report that variations existed of such magnitude that duplicate values were hardly to be expected. They back their contention with mathematical proof, since they subjected their results to the usual statistical analysis. In biological data, however, it is questionable whether implicit faith can be placed in such proof, for many factors enter into the simplest of biological phenomena, some of which are known, some are not. If, in addition to the known variables, one takes into consideration the fact that variables also exist which are either imperfectly known or are even unknown, then the mathematical interpretation is to a certain extent, invalidated. Wright and Thornton's paper seemed so convincing that their work was repeated by ourselves (3) with rather interesting results.

A single dilution of milk was prepared of such a bacteria content, as determined by the direct count, that plates would have 150 to 200 colonies. From this dilution 75 plates were poured, 15 at a time, numbering each plate consecutively as it was poured. After incubation and counting statistical analyses were made from which the usual constants were obtained. We found great variations, as did Wright and Thornton, but by numbering the plates, a fact was disclosed which led to still further work. It was ascertained that for the first few plates poured the count remained quite constant, but after this the colony count increased regularly. Figure 1 is a scatter diagram on one of these experiments, showing the colony count for each of the 75 plates as they were poured.

If each point on this graph were joined, a saw-toothed curve would be the result. The saw-teeth would represent the minor variations, which are doubtlessly inherent in the method, but superimposed upon these is a major trend, which certainly cannot be ascribed to the usual minor variations mentioned above.

Figure 2 is derived from the same data as the previous figure, but the running averages of 5 counts are plotted instead of

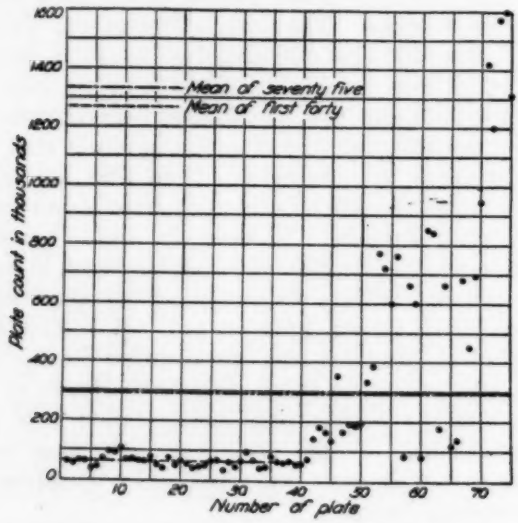

FIG. 1. DISTRIBUTION OF BACTERIA COUNTS THROUGH 75 PLATES SHOWING THE TENDENCY FOR THE COLONY COUNT TO INCREASE AS TIME INCREASES

each individual count. This overcomes the effect of the minor variations.

Figures 3, 4 and 5 are also given to show the constancy with which the increase is noted. Through these 5 plate averages a smooth curve can be drawn with very few non-conforming points. This curve indicates in a much better way than does figure 1 the increase in colony count which takes place when the dilution water is allowed to stand. At first thought, one would be tempted to ascribe this upward trend to growth, since the curve

itself is quite similar to a typical growth curve, but it should be pointed out at this time that this upward trend has been found in

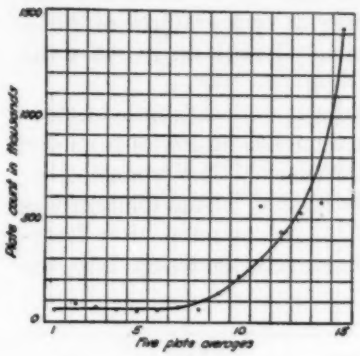

FIG. 2

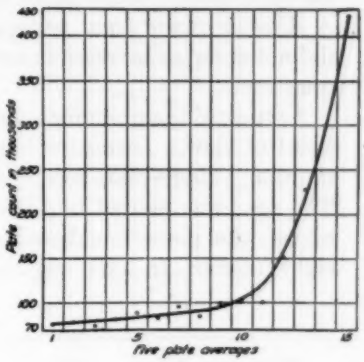

FIG. 3

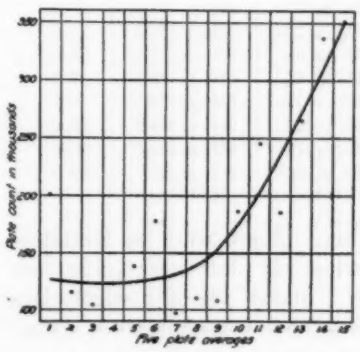

FIG. 4

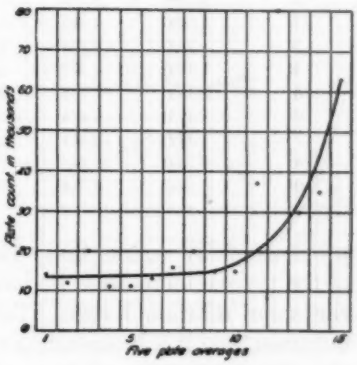

FIG. 5

FIG. 2. CURVE OBTAINED FROM PLOTTING FIVE PLATE AVERAGES OF COUNTS SHOWN IN FIGURE 1

FIGS. 3, 4 AND 5. CURVES SIMILAR TO THAT OF FIGURE 2 PLOTTED FROM OTHER SERIES OF PLATINGS

distilled water dilutions held in ice water. Growth would not ordinarily take place at this temperature and certainly not at such a rate as would be necessary to obtain the numbers found in

figure 1. The milk from which these organisms came did not itself show such an increase and in the milk the bacteria are in an environment much more suitable than in the dilution water. A dilution made from peptone, even at temperatures of 20°C. did not show an increase in two hours. Growth seems out of the question.

A study of the probable source of errors was made from another point of view. Instead of making a number of plates from one dilution, a single plate was poured from each of 50 dilution bottles. The agar was poured into the plate as soon as the dilution was added, the plates incubated at 37°C. and counted after forty-eight hours. In this way the time factor entering into the

TABLE 1

TRIAL	LOW	HIGH	MEAN	STANDARD DEVIATION	C.V.
1	1,500	3,700	2,436 ± 486	510 ± 34.3	20.9%
2	1,400	4,500	2,714 ± 649	680 ± 45.8	25.1
3	1,500	3,200	2,082 ± 429	450 ± 30.3	21.0
4	1,000	4,500	2,648 ± 611	640 ± 43.2	24.0
5	2,000	4,300	2,690 ± 448	470 ± 30.2	17.0
6	1,900	4,400	3,862 ± 334	350 ± 22.5	9.0
7	1,600	3,600	2,380 ± 363	380 ± 25.6	16.0
8	1,400	3,600	2,149 ± 410	430 ± 29.0	20.0
9	2,400	4,700	3,468 ± 448	470 ± 31.7	13.5

previous study was entirely lacking. There were no instances of the typical increase as noticed when the plots were made from the same dilution bottle. Table 1 shows the statistical data obtained, and it is interesting to note the low coefficients of variation obtained, although it must be remembered that the chance of error was increased by having each plate poured from a different dilution bottle. Since it might be of interest to graphically picture the results of this latter experiment, figure 6 is given to show the slight variation from the mean in each of those experiments. The abscissa is common to all trials, while the individual trials are indicated, each with its separate ordinate, which represents the count. From these data it was evident that the cause of the increase in colony count was associated with the time

of holding of the milk-water dilution. Something takes place in the dilution which causes an increase in the colonies on the plate.

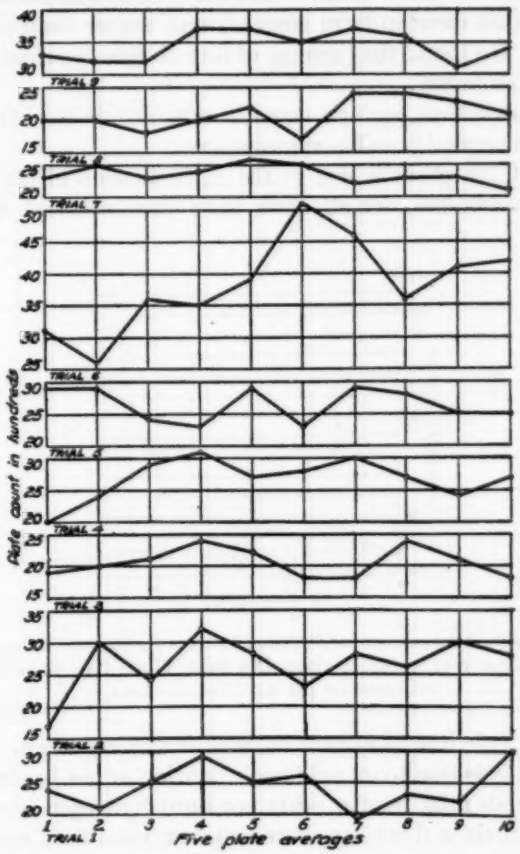

FIG. 6. FIVE PLATE AVERAGES FOR NINE SAMPLES OF MILK USING ONE DILUTION BOTTLE FOR EACH PLATE

That bacteria are present in milk as clumps or groups is a well known fact, recognized by all as a factor to be taken into consideration when the direct count is compared to the plate

count. This clumping does not refer to the strepto-form of cell grouping. Robertson (4) made a careful study of these clumps, finding that the plate count is really an estimate of the number of colonies that develop from such clumps, rather than from individuals. He found that groups of four to five occurred with the greatest frequency.

The reason that bacteria occur in this form is hard to explain. There are several possible explanations:

1. That the effect is due to the agglutination of the bacteria by the antibodies, which have been demonstrated time and again.

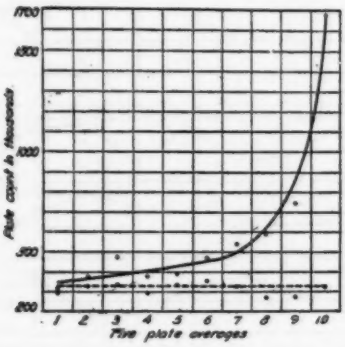

FIG. 7. FIVE PLATE AVERAGES OF COLONY COUNTS ON PLATES MADE FROM DISTILLED WATER (SOLID LINE) AND FROM PHOSPHATE BUFFER SOLUTION OF pH 6.6 (BROKEN LINE)

2. That it is due to certain charges on the organism.
3. That it is due to an acid agglutination of the bacteria.

Since fresh milk is of a fairly constant hydrogen ion concentration and since there is a more or less typical flora common to milk of this hydrogen ion concentration, this study was continued in an endeavor to discover what was the cause of the upward trend. The thought behind the newer work was that the dilution of the milk would change the pH of the milk, which in turn might lessen the forces holding the bacteria in the clumps.

With this in mind, two types of dilution water were prepared. One was distilled water, the other was a buffered solution, ad-

justed to pH 6.6 as suggested by Clark (5). Several samples of milk were used, dilutions being made as before, after a direct microscopic examination of the milk. Fifty plates were poured from each of the dilutions, the plates being numbered to deter-

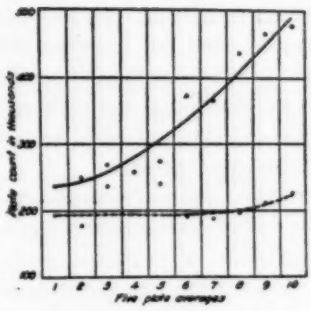

FIG. 8

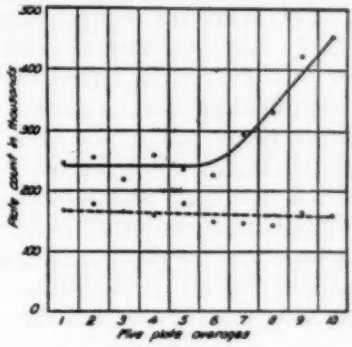

FIG. 9

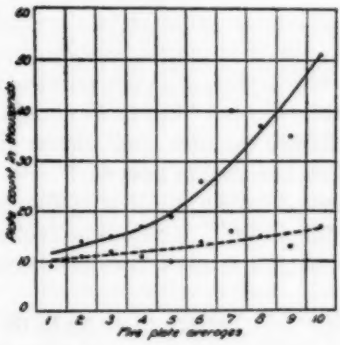

FIG. 10

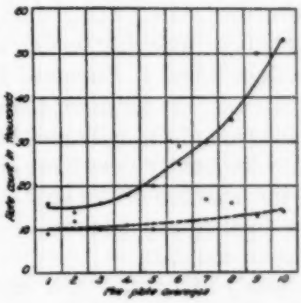

FIG. 11

FIGS. 8, 9, 10 AND 11. CURVES SIMILAR TO THAT OF FIGURE 7 PLOTTED FROM OTHER SERIES OF PLATINGS

mine the effect of holding time. The data are of considerable interest, and are shown in the figures 7 to 11. Here, as above, the running averages of 5 plates were plotted for the plates made from the two types of dilutions. The solid line represents the

counts derived from plates made from the distilled water, the dotted line represents the counts obtained from the plates made from the phosphate buffers of pH 6.6. This has been demonstarted many times during this study. At no time has the count in the buffer solution shown an appreciable rise, and at no time has the unbuffered solution failed to show the effect. As mentioned above, this upward trend is found in distilled water that has been kept in ice water, which would tend to disprove the contention that this is a growth effect. It further disproves the growth theory and at the same time substantiates the buffered solution theory. A 1 per cent peptone solution adjusted to pH 6.6 has been used with substantially the same results.

A statistical analysis of the data from which figure 7 was derived discloses that the plates made from the unbuffered dilution had a mean of 592,000 ± 269,000 and a coefficient of variation of 67 per cent, while those from the buffered solution had a mean of 310,000 ± 31,000 with a coefficient of variation of but 17 per cent.

It is quite evident that the milk-water dilution is a considerable factor in the plating of milk, and that more attention should be given it. It would be well to call attention at this point to the directions found in Standard Methods that "the work should be so planned that no more than fifteen minutes shall elapse after the dilution of the milk and before the agar is poured."

Too frequently does time elapse between dilution of the milk and the addition of the agar to the plates. If this time factor is more than fifteen minutes, it is easy to explain possible discrepancies in counts.

A study of figure 1 will explain this better. Let plate 60 be chosen for one of a pair of plates, with its colony count of 80,000. It would be well to recall that about an hour has elapsed from the time the dilution was made. If plate 59 is to be considered the duplicate of plate 60, its colony count is 600,000, while that of plate 61 is 850,000. The explanation for this in the light of the present study, is that plates 59 and 61 were made from less large clumps, while by chance plate 60 was seeded with clumps about the size originally found in the dilution bottle. (See plates 1–40.)

Attempts to actually see the dispersion of the clumps by means of staining were unsuccessful. The evidence is entirely circumstantial, that the dispersion effect is the correct solution of the phenomenon, but the evidence seems to be substantial.

An attempt was made to use bile to disperse the clumps to an even greater extent. This was unsuccessful.

CONCLUSION

It has been shown that:

The statistical studies upon the plate method are subject to invalidating effects.

A significant rise in colony formation takes place upon allowing the milk-water dilution to stand.

The addition of a phosphate or a peptone buffer of pH 6.6 prevents this.

This effect is due to dispersion of clumps.

The dilution bottle is of a hitherto unsuspected importance in the process of plating.

Standard Methods should be rigidly adhered to in respect to the time interval between making of the dilution and the addition of the agar to the plate.

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DISTRIBUTION AND GROWTH OF BACTERIA IN BUTTER*

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In 1927 it was shown by one of the authors that the number of moisture droplets in butter is very large, ranging from about 10 to 18 billions per gram of butter. In butter from ripened cream we seldom find more than 50 millions of microörganisms, in pasteurized sweet-cream butter sometimes only 10,000 are present in 1 gram of butter. Thus it is evident that not all moisture droplets in butter contain bacteria; the majority must be free from them; sterile drops are formed in the churning process. How small a percentage of the total moisture is infected may be seen from the following calculations and from one of our first experiments.

If we know the number and the size of the moisture droplets of the butter and the number of bacteria per cubic centimeter in the buttermilk, we can easily compute the percentage of the infected moisture. The distribution of the moisture in butter can be measured accurately enough. The method of Boysen (1) is as follows:

All moisture droplets are measured and counted directly under the microscope in a thin film of butter. They are divided into 12 groups, the first comprising all droplets up to 3μ in diameter, the next from 3 to 5μ, then 5 to 10μ, 10 to 15μ, 15 to 25μ, 25 to 35μ diameter and so on up to 100μ. Droplets larger than these cannot be seen and accurately counted under the microscope as a layer of butter thicker than 100μ or 0.1 mm. is not sufficiently transparent.

The groups from 0 to 15μ in diameter are counted in a thin layer of butter in special counting chambers (Zeiss) of 10μ depth at a magnification of 900 diameters (Zeiss eyepiece 15 $\times$ and objec-

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tive 60×, dry system). A net-micrometer in the eyepiece (fig. 1) allows one to measure each droplet and to count all droplets of a certain size in a given field. In the above-mentioned combination of lenses, the length of one part of the scale is exactly one micron, the largest square then holds 100 × 100 = 10,000 square microns, and as the depth of the film is 10 microns, the measured space is 100,000 cubic microns. Two fields in each of 10 preparations are examined in this way and the numbers are calculated for 1 gram of butter.

The larger drops are measured and counted at a magnification of 150 diameters (same eyepiece and objective 10×) in a counting-chamber of 100μ depth. Four of the largest squares are

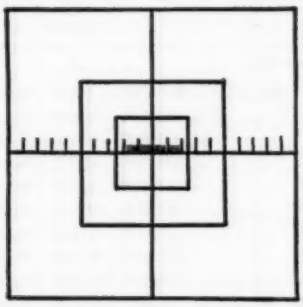

FIG. 1. NET-MICROMETER FOR EYEPIECE

examined in 10 preparations each, the total examined space thus being exactly 1 gram of butter.

The average volume of the droplets of each group has been determined, and by multiplying the number of droplets of each group with the corresponding average volume, the whole water volume is obtained. This volume means in the case of salted butter, practically also its weight. However, in unsalted butter, about 5 per cent of the computed water volume has to be added to change cubic centimeters into grams on account of the different densities.

The percentage of moisture in butter found in this way is usually smaller than the percentage found by chemical analysis.

This difference probably represents the drops larger than 100μ in diameter. There is some evidence for this in the fact that in some experiments in which butter was highly overworked this difference decreased to an amount which lies within the limits of error. Practically all moisture could be accounted for by this method; the percentage in the smaller droplets increasing continuously as the working process went on.

TABLE 1

Calculation of the amount of infected moisture in butter A

DIAMETER OF MOISTURE DROPLETS	AVERAGE VOLUME OF ONE DROPLET	NUMBER OF DROPLETS IN 1 GRAM OF BUTTER	VOLUME OF ALL DROPS IN 100 GRAMS OF BUTTER	PERCENTAGE OF INFECTED DROPS	GRAMS OF INFECTED MOISTURE IN 100 GRAMS OF BUTTER
μ	cubic microns		cc.		
0– 3	3.59	9,852,000,000	3.675	0.000,018	Under 1 mgm.
3– 5	33.5	253,000,000	0.893	0.000,168	Under 1 mgm.
5–10	221	39,000,000	0.905	0.001,100	Under 1 mgm.
10–15	1,020	7,000,000	0.751	0.005,010	Under 1 mgm.
15–25	4,190	360,000	0.159	0.021	Under 1 mgm.
25–35	14,100	90,000	0.133	0.070	Under 1 mgm.
35–45	33,500	32,000	0.112	0.168	Under 1 mgm.
45–55	65,500	24,000	0.165	0.328	0.001 gram
55–65	113,000	18,000	0.213	0.565	0.001 gram
65–75	180,000	6,000	0.113	0.900	0.001 gram
75–85	268,000	2,000	0.056	1.340	0.001 gram
85–95	382,000	2,000	0.080	1.910	0.002 gram
100 and more*			5.365		3.317 grams
Total.....		10,000,000,000	12.620		3.323 grams

* = extrapolated.

As the percentage for each of the larger groups becomes almost constant, it is possible to extrapolate the distribution of the drops larger than 100μ, and the results are rather plausible. In the most unfavorable cases the error of this method may be as high as 0.46 per cent of the butter weight. Table 1 gives the distribution of moisture in one of the samples of the first experiment, and table 2 shows the average distribution of moisture in German butter, and the method of extrapolation.

TABLE 2

Influence of the bacterial content of cream upon the amount of moisture remaining sterile in butter

DIAMETER OF DROPLETS	AVERAGE NUMBER OF MOISTURE DROPLETS PER GRAM OF BUTTER	PER CENT OF MOISTURE IN BUTTER	GRAMS OF MOISTURE INFECTED IN 100 GRAMS OF BUTTER IF THE BACTERIA COUNT OF BUTTERMILK AMOUNTS TO						
			1 billion	100 million	10 million	1 million	100 thousand	10 thousand	1 thousand
0– 3	11,068,000,000	3.97	0.014	0.001	0.000	0.000	0.000	0.000	0.000
3– 5	180,700,000	0.61	0.020	0.002	0.000	0.000	0.000	0.000	0.000
5–10	60,230,000	1.33	0.293	0.029	0.003	0.000	0.000	0.000	0.000
10–15	11,570,000	1.18	1.180	0.120	0.012	0.001	0.000	0.000	0.000
15–25	600,000	0.25	0.25	0.105	0.010	0.001	0.000	0.000	0.000
25–35	160,000	0.23	0.23	0.23	0.032	0.003	0.000	0.000	0.000
35–45	69,000	0.23	0.23	0.23	0.077	0.008	0.001	0.000	0.000
45–55	29,000	0.19	0.19	0.19	0.124	0.012	0.001	0.000	0.000
55–65	16,000	0.18	0.18	0.18	0.18	0.020	0.002	0.000	0.000
65–75	12,000	0.22	0.22	0.22	0.22	0.039	0.004	0.000	0.000
75–85	6,500	0.17	0.17	0.17	0.17	0.046	0.005	0.000	0.000
85–95	5,100	0.19	0.19	0.19	0.19	0.073	0.007	0.001	0.000
100	3,800	0.20	0.20	0.20	0.20	0.105	0.011	0.001	0.000
110	2,900	0.20	0.20	0.20	0.20	0.139	0.014	0.001	0.000
120	2,200	0.20	0.20	0.20	0.20	0.181	0.018	0.002	0.000
130	1,700	0.20	0.20	0.20	0.20	0.20	0.023	0.002	0.000
140	1,400	0.20	0.20	0.20	0.20	0.20	0.030	0.003	0.000
150	1,140	0.20	0.20	0.20	0.20	0.20	0.035	0.004	0.000
160	940	0.20	0.20	0.20	0.20	0.20	0.043	0.004	0.000
170	775	0.20	0.20	0.20	0.20	0.20	0.051	0.005	0.001
180	655	0.20	0.20	0.20	0.20	0.20	0.061	0.006	0.001
190	560	0.20	0.20	0.20	0.20	0.20	0.072	0.007	0.001
200	480	0.20	0.20	0.20	0.20	0.20	0.084	0.008	0.001
210	415	0.20	0.20	0.20	0.20	0.20	0.097	0.010	0.001
220	360	0.20	0.20	0.20	0.20	0.20	0.111	0.011	0.001
230	315	0.20	0.20	0.20	0.20	0.20	0.127	0.013	0.001
240	277	0.20	0.20	0.20	0.20	0.20	0.145	0.015	0.001
250	245	0.20	0.20	0.20	0.20	0.20	0.163	0.016	0.002
260	218	0.20	0.20	0.20	0.20	0.20	0.184	0.018	0.002
270	195	0.20	0.20	0.20	0.20	0.20	0.200	0.021	0.002
Moisture infected			6.777	5.277	4.628	3.628	1.489	0.147	0.011
Moisture sterile			5.583	7.083	7.732	8.732	10.871	12.213	12.349
Total moisture		12.36	12.36	12.36	12.36	12.36	12.36	12.36	12.36
Per cent of total moisture sterile			45.2	57.4	62.5	71.0	88.0	99.0	(100)

The computation of the amount of infected moisture in butter is shown in table 1. Butter A was neither washed nor salted and its plasma, i.e. the fat-free part of the butter, must be of the same approximate composition as the corresponding buttermilk and, of course, must have the same approximate bacterial count which was in this case 50,000 per cubic centimeter, the cream being pasteurized and unripened. One gram of the butter contained 9,852,000,000 droplets of the smallest group, under 3μ in diameter which amounted in total to 0.0368 gram of moisture. As this moisture contained 50,000 bacteria per cubic centimeter, there are in these smallest droplets $0.0368 \times 50{,}000 = 1{,}840$ bacteria. This simply means that not more than 1,840 out of 9.8 billion droplets can be infected. The rest, 99.99,998 per cent, must be sterile.

As the size of the droplets increases, the amount of the moisture infected also increases. This is shown in table 1 where the calculation has been carried out for all 12 groups, including the extrapolation. The general formula for the percentage of infected moisture is $\frac{W \cdot N}{D}$, in which W is the amount of water of each group in 100 grams of butter, N is the bacterial count of 1 cc. moisture in the butter and D is the number of drops of the group in question. Then the amount of the infected moisture in 100 grams of butter is $\frac{W \cdot W \cdot N}{100 \cdot D}$, or as $\frac{W}{100 \cdot D}$ equals the average volume of that group: $W \cdot N \cdot$ volume.

In our sample of butter, 3.32 grams of moisture in 100 grams of butter are infected, i.e., 26.4 per cent of the total moisture content. In table 2, this calculation has been carried out for various bacterial counts in the buttermilk on the basis of an average moisture distribution as found in German butter. It shows that in butter from very sour cream, about 50 per cent of the total moisture is sterile, while in well pasteurized sweet-cream butter almost 99 per cent is free from bacteria. All practical cases probably lie between these two limits.

It does not seem very reasonable and probable that in churning a highly infected cream, so large a part of the new product be-

comes sterile. Yet it is a fact, as these calculations are based upon well established data only. And it does not seem so surprising if we consider that even in a full grown culture of *Streptococcus lactis* with a billion bacteria per cubic centimeter every cell has still a living space of 1000 cubic microns, while the smallest moisture droplets in butter average only 3.59 cubic microns.

This is also evident by the low count of bacteria in butter which has remained unexplained so far; the largest number of bacteria per gram of butter has been recorded by Orla-Jensen (5) to be 59,000,000, by Teichert (10) 22,000,000, by Lorenz (4) 49,000,000, by Schmidt (9) 27,000,000, by Sayer, Rahn and Farrand (8) 26,000,000. The lactic acid organisms may multiply in milk to 1,000,000,000 cells per cubic centimeter, as may also the colon organisms, *Pseudomonas fluorescens* and other putrifiers. So it would be expected that in butter with 15 per cent of moisture, we should find about 15 per cent of 1,000,000,000, i.e., 150,000,000 bacteria per gram. The fact that never more than 59,000,000 have been observed, is an indication that conditions of growth in butter must be different from those in milk. The computation of the bacteria-free moisture droplets gives a simple explanation for this difference.

The question arises as to how this uneven distribution of bacteria in the moisture influences the keeping quality of the butter. According to the two conceptions of the structure of butter there are two possibilities [Rahn (6)]. The theory of the inversion of phases claims that all water droplets are entirely separated from each other, the fat being the continuous phase. In this case decomposition of butter could take place only to the extent in which its moisture is infected. It is not entirely impossible however that some slight exchange might take place through the fat, as, e.g., lactic acid formed in the larger infected drops may diffuse to the sterile ones because lactic acid is slightly fat-soluble, thus giving the bacteria a new chance to ferment more lactose. According to the foam theory of the churning process all drops are connected by a very thin network consisting of the membranes of fat globules which are thick enough to serve as diffusion channels but which are too narrow to let bac-

teria pass, the smallest of which would be at least 10 to 20 times as large as these connecting layers. In this case a slow diffusion of the products would be possible, thus causing butter to be decomposed to a higher degree than would be expected according to the percentage of infection. The two structural possibilities are illustrated in the textbook of Rahn and Sharp (7, p. 111).

To decide between the two possibilities, comparisons have been made between butter and true solidified emulsions of skimmilk in butterfat. The other questions dealt with here are those of the influence of washing, working and salting the butter. Our preliminary experiments are omitted as our efforts to separate the small from the large drops in butter failed.

In all series of experiments the formation of acid was used as a measure of bacterial decomposition and it was determined by titrating with N/14 NaOH with phenolphthalein as indicator. In the first two experiments the hydrogen-ion concentration was determined also but was later omitted as the exact amount of lactic acid cannot be determined in this way in such highly buffered solutions. In all butter samples the distribution of the moisture droplets was determined according to the method described above. Furthermore, the bacterial content was determined both in the butter and in the buttermilk. For the determination of the acid, about 100 grams of butter were melted at 70° to 80°C. until the liquid fat had separated entirely from the plasma, the plasma was then mixed before titration.

In experiment I a quantity of pasteurized cream was divided into four parts and churned separately in the same churn. One part was neither washed nor salted, another washed but not salted. The two other parts were salted, one of them being washed. Of each of these four portions one-half was normally worked whereas the rest was overworked, thus giving eight different samples of butter which were stored, together with a sample of the buttermilk, under the same conditions at room temperature.

Table 3 shows the distribution of the water droplets in these eight samples and the amount of infected moisture as calculated in the described way. Unfortunately, a finer distribution of the

water by overworking was obtained only in the first case. In the other samples the number of small droplets was increased but so were the larger drops because more water had been worked into the butter during the overworking process. Thus the percentage of infected moisture appears a little too high because the increase in moisture after overworking did not come from the buttermilk but from the washwater which did not contain as many bacteria.

TABLE 3

Distribution of moisture in the samples of experiment I

TREATMENT OF THE BUTTER			SAMPLE	AMOUNT OF MOISTURE IN DROPLETS OF:			TOTAL MOISTURE	INFECTED MOISTURE
				0–15μ	15–100μ	Over 100μ diameter		
				per cent	*per cent*	*per cent*	*per cent*	*per cent*
Unsalted	Unwashed	Normally worked	A	6.22	1.03	5.37	12.62	26.4
		Overworked	B	8.13	0.93	4.04	13.10	17.0
	Washed	Normally worked	C	5.95	1.12	4.95	12.02	23.0
		Overworked	D	6.84	0.81	5.57	13.22	30.0
Salted	Unwashed	Normally worked	E	5.47	2.09	3.71	11.27	4.8
		Overworked	F	6.10	0.86	5.69	12.65	32.3
	Washed	Normally worked	G	5.88	1.52	3.50	10.90	7.0
		Overworked	H	5.77	1.32	5.31	12.40	22.0

In the case of samples A and B, unsalted and unwashed butter, the composition of the control plasma is exactly like that of the buttermilk. It is therefore possible to compare decomposition directly. The formation of acid in the plasma of the butter samples and in the buttermilk should be the same if the changed physical conditions of the moisture in the butter had no influence. Table 4 shows that the buttermilk sours much faster than does the equal amount of moisture present in the butter. For exact comparison we have to consider the *increase* in acidity instead of the total acidity. In three days the buttermilk increased its

acidity 0.7266 per cent, while the corresponding increases in the butter samples A and B were only 0.3086 and 0.1736 per cent (calculated as lactic acid). This is about 42 and 24 per cent of the increase in the buttermilk, which must be considered as

TABLE 4

Acid formation in the plasma of the butter samples of experiment I

	NOT SALTED				SALTED				BUTTERMILK
	Unwashed		Washed		Unwashed		Washed		
	Normally worked	Overworked	Normally worked	Overworked	Normally worked	Overworked	Normally worked	Overworked	
	A	B	C	D	E	F	G	H	
	Infected moisture in percentage of total moisture in samples								
	26.4	17.0	23.0	30.0	4.8	32.3	7.0	22.0	100
Acidity in percentage of lactic acid									
After 0 day	0.2508	0.2508	0.1222	0.1029	0.1993	0.1998	0.1190	0.1093	0.2572
After 1 day	0.2926	0.2765	0.1610	0.1180	0.2251	0.2251	0.1670	0.1225	0.8745
After 2 days	0.3729	0.3022							0.9774
After 3 days	0.5594	0.4244	0.1870	0.1542	0.2000	0.2775	0.1290	0.1227	0.9838
After 11 days			0.2260	0.1870	0.2630	0.2380	0.1740	0.1290	
Increase in acidity; percentage of lactic acid									
After 1 day	0.0418	0.0257	0.0388	0.0151	0.0258	0.0258	0.0480	0.0132	0.6173
After 2 days	0.1221	0.0514							0.7202
After 3 days	0.3086	0.1736	0.0648	0.0513	0.0007	0.0782	0.0100	0.0134	0.7266
After 11 days			0.1038	0.0841	0.0637	0.0380	0.0550	0.0197	
pH									
After 0 day	5.97	5.95	6.39	6.41	5.45	5.50	5.75	5.80	5.90
After 1 day	5.72	5.70	6.29	6.40	5.51	5.50	5.74	5.75	4.50
After 2 days	5.48	5.65	6.18	6.36	5.33	5.55	5.38	5.80	4.15
After 3 days	5.52								4.20
After 11 days			5.51	5.56	5.51	5.63	5.55	5.78	

the highest possible increase. The acid formation is less in the butter than in the buttermilk, yet it is higher than would be expected. The percentage of infected moisture is 26.4 per cent in the case of butter A and 17 per cent in the case of butter B

which would account only for about two-thirds of the actually observed increase. Therefore fermentation does not seem to be limited to the infected drops only.

The other samples show the effect of washing and salting; this is dealt with later. They cannot be compared directly with A and B and the buttermilk because the composition of their plasma is not the same as that of the free buttermilk.

In order to get complete comparisons, the experiment was repeated; this time the pasteurized cream was inoculated with a very small amount of starter, and instead of four samples only

TABLE 5

Distribution of the moisture in the samples of experiment II

TREATMENT OF THE BUTTER			SAMPLE	PERCENTAGE OF MOISTURE IN DROPS WITH A DIAMETER OF:			TOTAL MOISTURE	INFECTED MOISTURE
				0–15μ	15–100μ	Over 100μ		
				per cent			*per cent*	*per cent*
Unsalted	Unwashed	Normally worked	I	7.72	1.11	1.75	10.58	7.7
	Washed	Normally worked	II	7.65	0.61	4.09	12.35	29.2
		Overworked	III	7.92	0.49	6.55	14.96	41.0
Salted	Washed	Normally worked	IV	5.01	1.38	5.38	11.77	36.8
		Overworked	V	7.12	0.67	5.48	13.27	31.5

one was left unwashed. In order to obtain a "buttermilk" comparable with the plasma in the washed and salted, and also in the washed and unsalted butter, several pounds of each of these samples were melted at a temperature not over 40°C. and the plasma separated. This liquid was approximately comparable with the moisture present in the respective butter samples in chemical as well as in bacteriological respects. All samples were printed and immediately brought into a room at 22°C. where they were kept together with the buttermilk samples. The buttermilk contained about 200,000 bacteria per cubic centimeter. The distribution of the moisture droplets is given in table 5 and the acid formation in table 6.

Unfortunately, also in this case, overworking did not decrease the moisture content and did not make the distribution finer, thus giving again a wrong number for the percentage of infected

TABLE 6

Acid formation in the plasma of the butter samples of experiment II

	NOT SALTED			SALTED		BUTTERMILK	PLASMA OBTAINED FROM SAMPLES	
	Unwashed	Washed		Washed				
	Normally worked	Normally worked	Overworked	Normally worked	Overworked			
	I	II	III	IV	V		II + III	IV + V
	Infected moisture in percentage of total moisture in samples							
	7.7	29.2	41.0	36.8	31.5	100	100	100
Acidity in percentage of lactic acid								
After 0 day.....	0.4050	0.2060	0.1675	0.2180	0.1994	0.4960	0.3540	0.3540
After 2 days.....	0.3870	0.2830	0.2325	0.2380	0.2265	0.7735	0.5670	0.3355
After 4 days.....	0.4960	0.3280	0.2710	0.2900	0.2610	0.8770	0.6570	0.3415
After 7 days.....	0.5210	0.3600	0.2970	0.3180	0.2610	0.9250	0.8380	0.3280
After 32 days.....	0.6620	0.4735	0.4370	0.3000	0.2950			
After 64 days.....	0.6623	0.5401	0.4951	0.3215	0.2765			
Increase in acidity; percentage of lactic acid								
After 2 days.....	(−0.0180)	0.0770	0.0650	0.0200	0.0271	0.2775	0.2130	(−0.0185)
After 4 days.....	0.0910	0.1220	0.1035	0.0720	0.0616	0.3810	0.3030	(−0.0125)
After 7 days.....	0.1160	0.1540	0.1295	0.1000	0.0616	0.4290	0.4840	(−0.0260)
After 32 days.....	0.2570	0.2675	0.2695	0.0820	0.0956			
After 64 days.....	0.2573	0.3341	0.3276	0.1035	0.0771			
pH								
After 0 day.....	4.95	5.30	5.40	4.95	5.02	5.02	5.11	5.00
After 2 days.....	5.06	4.89	5.11	4.63	3.67	4.11	4.21	4.94
After 4 days.....	4.92	5.14	5.11	4.94	5.01	4.08	4.07	5.05
After 7 days.....	4.86	5.18	4.96	5.03	5.03	4.20	3.84	3.44
After 32 days.....	4.58	4.72	4.50	5.48	5.95			
After 64 days.....								

moisture. These results will be discussed later. The three unsalted samples show a distinctly slower decomposition than the buttermilk or the separated plasma. But in all cases

finally the amount of acid found in butter is 62 to 69 per cent of that of the corresponding free moisture, while the percentage

TABLE 7

Distribution of moisture in the butter samples of experiment III

SAMPLES	PERCENTAGE OF MOISTURE IN DROPLETS WITH A DIAMETER OF			TOTAL MOISTURE	INFECTED MOISTURE
	0–15μ	15–100μ	Over 100μ		
				per cent	*per cent*
Normally worked	8.29	0.91	4.77	13.97	37.6
Thoroughly worked	9.08	0.68	4.21	13.97	33.4
"Creamed"	9.95	0.52	3.50	13.97	27.2

TABLE 8

Acid formation in the plasma of the butter samples of experiment III

	NORMALLY WORKED	THOROUGHLY WORKED	"CREAMED"	PLASMA OBTAINED BY MELTING
	Sample N	Sample O	Sample C	
Acidity in percentage of lactic acid				
After 0 day	0.055	0.055	0.055	0.055
After 1 day				0.296
After 2 days	0.129	0.071	0.064	0.456
After 5 days				0.520
After 6 days	0.238	0.148	0.071	0.540
After 8 days				0.559
After 12 days	0.289	0.161	0.084	1.138
After 24 days	0.335	0.196	0.116	1.010
After 42 days	0.412	0.232	0.116	
After 60 days	0.472	0.386	0.180	1.090
Increase in acidity; percentage of lactic acid				
After 2 days	0.074	0.016	0.009	0.401
After 6 days	0.183	0.093	0.016	0.485
After 12 days	0.234	0.106	0.029	1.083
After 24 days	0.280	0.141	0.061	0.955
After 42 days	0.357	0.177	0.061	
After 60 days	0.417	0.331	0.125	1.035

of infected moisture amounts only to 8 to 41 per cent. Here we have the same result as in the first experiments; more droplets

are decomposed than are infected. Both of the salted samples, strange to say, show a slight increase in titrable acidity and a decrease in hydrogen-ion concentration. The comparable moisture also shows a decrease in titrable acidity. However interesting this behavior may be, it does not help to solve the problem of the bacterial distribution and therefore it may be left out of consideration.

Another experiment was made in order to get an overworked butter without an increased moisture content. Pasteurized sweet cream was inoculated with lactic acid bacteria and churned at once. Of the resulting butter one part was worked normally while another part was overworked and an equal portion brought to a creamy consistency in a mortar with a pestle. All samples were treated in the same way and again a certain amount of free plasma was obtained by melting. All three samples being alike, save the mechanical treatment, one plasma sample was sufficient for all three. The results are shown in tables 7 and 8.

THE MEANING OF THE STERILE MOISTURE

In all experiments in which a noticeable decomposition of the plasma took place, the acid formation went further than would be expected from the amount of infected moisture. The seven samples of unsalted butter of the first two experiments give the most information on this point. A comparable moisture existed for all except samples C and D. Here the undiluted buttermilk was taken for the calculations, though the butter was washed. But acid formation is slowed down only very little by diluting even with equal amounts of water. (See the plasma in table 6.)

In table 9 the increase in acidity in 100 cc. of moisture *in* the butter is calculated in percentages of the same moisture *outside* of the butter. In each case, 100 is the number which would be obtained if the liquid was not distributed in the form of small droplets but was present as the continuous phase, as buttermilk or diluted buttermilk.

If there were no possibility of exchange between infected and sterile droplets, only as much acid could be formed in the butter

as corresponds to the percentage of infected plasma. In the case of butter A, the amount of acid in the butter should, at any time, be 26.4 per cent of the amount of acid in the buttermilk. The table shows that this is not the case. In the first days, however, this holds fairly true. In the unwashed samples this limit is exceeded already on the third day, while in the washed samples this does not take place until after the twelfth day.

In the washed samples, however, we have to consider that the value for the infected moisture is too high. During the washing

TABLE 9

Acidity of the plasma in percentage of total acidity possible

SAMPLE	INFECTED PLASMA IN PERCENTAGES OF TOTAL	ACIDITY OF PLASMA IN PERCENTAGES OF TOTAL ACIDITY POSSIBLE, ON THE FOLLOWING DAYS												
		1	2	3	4	6	7	11	12	24	32	42	60	64
Unwashed butter														
A	26.4	7	17	42										
B	17.0	4	7	24										
I	7.7		0		24		27				62			62
Washed butter														
C	23.0	6		10				14						
D	30.0	2		6				12						
II	29.2		36		41		32				55			69
III	41.0		31		34		27				56			68
N	37.6		18			38			23	27		35	41	
O	33.4		4			19			10	14		17	32	
C	27.2		2			3			3	6		6	12	

process, buttermilk with its high bacterial count is replaced by water with a low count. Yet this error is not so great that it can explain the slower acid formation.

Sooner or later, in every butter, more acid is formed than corresponds to the infected plasma. Some kind of exchange must then take place between the infected and the sterile droplets. There are again the two possibilities. If all droplets are connected with each other as Rahn (6) believes this would mean a slow diffusion. Rahn found that salt from a 25 per cent brine

diffused 15 mm. into unsalted butter in three months. i.e., 15,000μ in ninety days, or 167μ in one day, or 7μ per hour. The average distance between the water droplets amounts to about 5μ[1] so that a rather complete exchange seems possible, though, of course, a 25 per cent brine diffuses much faster than a 0.1 per cent lactic acid.

However, this conception of Rahn of the structure of butter is not generally accepted. Hunziker (3) assumes that all water droplets lie separately in butter. In this case, an exchange would be possible only if the lactic acid could diffuse through the continuous fat walls. This is not quite impossible as lactic acid is soluble in fat. A simple experiment was tried to decide this question. Solutions of 5 and 1 per cent lactic acids were kept separated from pure water by a 5 mm. film of butterfat. After more than two months no trace of acid could be found in the outside water.

At the same time this problem was worked on from another point of view. Artificial "butter" was prepared by emulsifying skimmilk, inoculated with lactic acid bacteria, in melted, filtered butterfat. It is rather certain that in such an emulsion all water droplets are single without any connections between each other. The emulsions, churned in a glass churn, were solidified rapidly with cold water and were then treated just like the butter samples of the previous experiments.

In the first experiment the number of lactic acid bacteria was too low, thus causing only 0.66 per cent of the plasma to be infected. This emulsion did not show any change at all until finally it molded. In a second experiment one emulsion was made to contain more bacteria than the other. This time, however, the emulsions were rather coarse and thus the calculations of the infected plasma are more inaccurate than usual. Yet the data in table 10 show that after ten days, and in one case after sixteen days, no further increase in decomposition takes place for some

[1] After the formula: $X = d\left(\sqrt[3]{\frac{74.04}{W}} - 1\right)$ in which d is the average diameter of the water droplets and W the water content in 100 grams of butter (Rahn and Sharp (7, p. 178)).

time. The irregularities in the lower part of the table were found to be due to the development of high acid producing organisms one of which formed, in milk, 1.9 per cent of lactic acid in four weeks. It seems, therefore, correct to neglect the figures for the sixty-fourth day because the three preceding data are as constant as can be expected with this method of determination. The con-

TABLE 10

Solidified emulsions of skimmilk in butterfat

A. Distribution of moisture

SAMPLE	NUMBER OF BACTERIA IN 1 CC. OF THE PLASMA	PERCENTAGE OF MOISTURE IN DROPLETS OF			TOTAL PERCENTAGE OF MOISTURE	PERCENTAGE OF INFECTED MOISTURE
		0–15μ	15–100μ	over 100μ		
E I	13,500	2.32	3.33	8.85	14.50	4.4
E II	740,000	2.12	2.89	9.49	14.50	63.4

B. Acid formation in percentage of lactic acid

	TOTAL ACIDITY				INCREASE IN ACIDITY			
	E I		E II		E I		E II	
	Emulsion	Plasma	Emulsion	Plasma	Emulsion	Plasma	Emulsion	Plasma
After 0 days.....	0.116	0.142	0.119	0.155				
After 2 days.....	0.142	0.650	0.213	0.760	0.026	0.508	0.094	0.605
After 4 days.....	0.161	0.785	0.200	0.875	0.045	0.643	0.080	0.720
After 10 days.....	0.318	0.900	0.357	0.978	0.202	0.758	0.238	0.823
After 16 days.....	0.446	0.926	0.569	0.945	0.330	0.784	0.450	0.790
After 32 days.....	0.320	0.910	0.568	1.350	0.204	0.768	0.449	1.195
After 64 days.....	1.440	1.800	1.100	2.070	1.324	1.658	0.981	1.915

stancy as such is perhaps the best proof for the absence of any diffusion processes in the emulsions.

The decomposition in the emulsions reaches 26 and 56 per cent of that of the corresponding free plasma, these figures lie in one case way above, in the other a little below the computed values. The calculations for the percentage of infected moisture are not so accurate in the first emulsion because of the low bacterial count (13,000 per cubic centimeter) and the very uneven and coarse distribution of the moisture droplets. Emulsion II

seems to indicate that in true emulsions the decomposition does not exceed the amount of infected moisture.

THE INFLUENCE OF WASHING THE BUTTER

Not until lately has it been possible to sufficiently explain the good effect of the washing of butter on its keeping quality. The chemical analyses of Hittcher (2) show that hardly half of the lactose and less than one fourth of the curd can be washed out. Yet in milk, diluted to two volumes, all decomposition processes occur almost as fast as they do in undiluted milk. Dilution as such does not give an explanation for the better keeping of washed butter.

Rahn and Sharp (7, p. 117) give the following physical explanation for it: The smallest moisture droplets in butter originate during the churning process, they simply fill the intermediate spaces between the fat globules and they therefore contain pure buttermilk. These droplets are entirely enclosed in the butter-granules and do not come in contact with the washwater at all. From the outside of the butter-granules, however, the washwater removes all milky parts very thoroughly, since butter is usually washed until the drain water is clear. The water incorporated during the washing and working processes forms the larger drops. In butter two entirely different kinds of moisture droplets are present, namely, small droplets of buttermilk which are mostly sterile because of their minute size, and large drops which consist of almost pure water and which contain a mixture of bacteria from the cream and from the water. Since all calculations have shown that most of the bacteria are present in the large drops (see tables 1 and 2) the result of washing is that the food is present in the small droplets and the bacteria in the large ones, thus the bacteria being separated from their food. For this reason, protection of the butter by washing is so efficient, provided, of course, that the washwater is good. If the water contains lipolytic bacteria, e.g., *Ps. fluorescens*, the danger is greater, for in the large drops not enough lactose is present to allow of a strong acid formation which might retard lipolysis.

The assumption of Rahn and Sharp seems to be proved by these experiments. In table 11 are shown the increases in acidity in three samples of unwashed butter as compared with the washed control samples. All of these samples were unsalted.

The first two pairs show how acid formation is retarded in the washed samples. Butter II and III do not show this decrease, probably because of an incorrect determination of the initial acidity in sample I; for this butter shows a decrease in acidity after two days which seems quite improbable. The increases in acidity after the second day are:

In sample I	0.1090	0.1340	0.2750	0.2753
In sample II	0.0450	0.0770	0.1905	0.2571

TABLE 11

Increase in acidity in washed and in unwashed butter

	AFTER 1 DAY	AFTER 2 DAYS	AFTER 3 DAYS	AFTER 4 DAYS	AFTER 7 DAYS	AFTER 11 DAYS	AFTER 32 DAYS	AFTER 64 DAYS
A, unwashed	0.0418	0.1221	0.3086					
C, washed	0.0388		0.0648			0.1038		
B, unwashed	0.0257	0.0514	0.1736					
D, washed	0.0151		0.0513			0.0841		
I, unwashed		(−0.0180)		0.0910	0.1160		0.2570	0.2573
II, washed		0.0770		0.1220	0.1540		0.2675	0.3341

This error can also be eliminated by calculating the final acidity in butter I and II in percentages of the final acidity of the corresponding free moisture:

Acid formation in percentages of that in the free plasma

Butter I, unwashed	82	53	57	57	72	72
Butter II, washed	59	48	50	43	56	64

In all cases the unwashed sample soured more than the washed butter.

This influence of washing can be shown still in another way. Table 9 shows that the unwashed butter up to the fourth day has already soured more than would be expected according to the

infected plasma. In the case of washed butter this occurs only after the eleventh day. Butter is protected very much against bacterial decomposition by washing.

The salted samples do not prove very much about the washing as in all cases the increase in acidity was very slight and as these differences fall mostly within the limits of error.

THE INFLUENCE OF WORKING

In most of our experiments normally worked butter is compared with overworked butter. The purpose of overworking was to get a finer moisture distribution but unfortunately this was not obtained in all cases because in some experiments (D, III, and V) water had been incorporated during the overworking process. This, of course, was present in form of larger drops which, however, did not consist of buttermilk but of water. Table 12 shows that in almost every case the overworked sample soured more slowly than did the normally worked check.

The lower part of the table shows the increase in acidity of the overworked butter in percentages of the acid increase of normal butter. These figures give us a good idea of the effect of working on protection of butter against bacterial decomposition. Only six out of the 34 figures lie above 100 and in these, the overworked butter soured faster than the normal samples. Of these six samples, four are within the limits of experimental error since only very small quantities of acid were present in one to two days old salted butter. Only samples II and III remain as exceptions; in these two samples, acid formation was about equal, especially towards the end of the experiment.

Apparently there is no relation between the inhibitive effect or overworking on bacterial action and the amount of plasma infected. The table even shows that in many cases the distribution of moisture droplets has become coarser through the incorporation of new water. The calculations, therefore, are wrong; for the newly incorporated water contained much less bacteria than did the original moisture in the butter. In the case of washed

butter, therefore, every calculation of infection shows too high figures.

As a result of these comparative experiments we may say that the keeping quality of the butter is increased by more working. The finer the distribution of the moisture droplets, the less and more slowly is butter decomposed by bacteria. Heavy working of butter not only results in a better control of the moisture content but also in the improvement of keeping quality from a bacteriological viewpoint.

The present experiments show a decrease of the acid production by working in a degree which cannot be accounted for by the finer distribution of the moisture droplets alone. If we assume that all water droplets are connected with each other, there is still another possible explanation. It may be assumed that by overworking the connections between the moisture droplets are broken up more and more, thus decreasing or even preventing diffusion. Experiment III shows a decrease in decomposition going somewhat parallel with the degree of working and the percentage of infected moisture, as is shown here:

Final acidity in per cents of the acidity in the buttermilk, and per cents of infected moisture in the samples of experiment III

	N (NORMALLY WORKED)	O (THOUROUGHLY WORKED)	C ("CREAMED")
Acidity after 60 days	41	32	12
Per cent of moisture infected	37.6	33.4	27.2

This may be explained by the assumption stated above, but an experiment showed that there was practically no difference in the time and speed of salt-dissolution in these three samples and that finally all moisture droplets were attracted by the salt. Another point may enter here which would also account for the greatly decreased acid formation. It is possible that during the working process some bacteria become entirely separated from all water, i.e. they may be distributed through the fat. These bacteria could not cause any further decomposition.

TABLE 12

Increase in acidity in normally worked butter and in overworked butter

NUMBER	MOISTURE CONTENT	PER CENT OF PLASMA INFECTED	AFTER 1 DAY	AFTER 2 DAYS	AFTER 3 DAYS	AFTER 4 DAYS	AFTER 6 DAYS	AFTER 7 DAYS	AFTER 11 DAYS	AFTER 12 DAYS	AFTER 24 DAYS	AFTER 32 DAYS	AFTER 42 DAYS	AFTER 60 DAYS	AFTER 64 DAYS
A	12.6	26.4	0.0418	0.1221	0.3086										
B	13.1	17.0	0.0257	0.0514	0.1736										
C	12.2	23.0	0.0388		0.0648				0.1038						
D	13.2	30.0	0.0151		0.0513				0.0841						
E	11.3	4.8	0.0258		0.0007				0.0637						
F	12.7	32.3	0.0258		0.0782				0.0380						
G	10.9	7.0	0.0480		0.0100				0.0550						
H	12.4	22.0	0.0132		0.0134				0.0197						
II	12.4	29.2		0.0770		0.1220		0.1540				0.2675			0.3341
III	15.0	41.0		0.0650		0.1035		0.1295				0.2695			0.3276
IV	11.8	36.8		0.0200		0.0720		0.1000				0.0820			0.1035
V	13.3	31.5		0.0271		0.0616		0.0616				0.0956			0.0771
N	14.0	37.6		0.074			0.183			0.234	0.280		0.357	0.417	
O	14.0	33.4		0.016			0.093			0.106	0.141		0.177	0.331	
C	14.0	27.2		0.009			0.016			0.029	0.061		0.061	0.125	

Overworked in percentage of normally worked butter

A:B	62	42	56										
C:D	39		80				81						
E:F	100		(1,100)				60						
G:H	27.5		134				36						
II:III		84		120		83				101			98
IV:V		135		86		62				117			74
N:O		22			51			45	50		50	79	
N:C		12			9			12	22		17	30	
Average.....	57	59	(90)	103	30	73	59	29	36	109	34	55	86

SALTING

No new knowledge is gained from the experiments with salted butter. They simply confirm that the salt, in the concentrations used, almost entirely inhibits acid formation.

Only for samples IV and V, a truly comparable free plasma had been obtained. (See table 6.) Here no acid formation was observed at all, but a very slight decrease in acidity. Whether this was of biological origin or not, was not determined. The corresponding butter samples showed a slight increase in titrable acidity but a decrease in hydrogen ion concentration. This behavior is difficult to explain. In the salted samples: E, F, G, H of the first experiment the increase in titrable acidity was smaller, the pH remained constant.

One might be tempted to compare the condition of the moisture in salted butter with that in washed butter and to assume that there are two different kinds of moisture droplets: Large, salt containing drops and small droplets more or less free from salt. But this is not very probable as Boysen (1) has shown by micromotion pictures that the salt crystals attract all moisture droplets in their neighborhood. This attraction which always occurs in butter, but not in true solidified emulsions of skimmilk in butterfat, is an important proof for the direct connection of all moisture droplets in butter. Yet 98 per cent of butter salt ordinarily are crystals under 0.75 mm. in diameter, and 30 to 60 per cent are under 0.5 mm. That means that there is one salt crystal to about three to five millions of water droplets in the case of normally salted butter. Some of the most distant droplets might escape the influence of salt, but the amount of this moisture is probably negligible and only the concentration of the salt in the larger drops would be increased a little. Furthermore, the salt crystals attract the moisture droplets only; the bacteria must remain at their old places, they become desiccated, so to speak.

However, the most effective part of salt-action is the concentration of the brine which amounts to the following percentages:

	SAMPLE E	SAMPLE F	SAMPLE G	SAMPLE H	SAMPLE IV	SAMPLE V
Per cent of salt.....	13.0	11.8	8.6	8.6	10.0	10.4

The relatively low salt concentration of 10 per cent is sufficient to almost entirely inhibit bacterial fermentation.

SUMMARY

1. The authors believe to have established the fact that a considerable portion of the moisture of cream becomes sterile in churning by being divided up into very small droplets. Even in sour cream butter there are more than 100 droplets to one bacterium. In this case about 40 per cent of the moisture, mostly the smaller drops, will become sterile; with pasteurized sweet cream butter more than 80 per cent of the moisture remain free from bacteria.

2. The amount of moisture shut off from bacteria depends upon two factors: i.e., the number of bacteria in the cream at the moment of churning and the degree of dispersion of the moisture in butter. In overworked butter more moisture is made sterile.

3. All experiments show uniformly that the formation of acid in the moisture within the butter is much slower than if the same amount of moisture is freed from fat and in the form of a continuous liquid. But it seems that the percentage of acid formed in butter slowly increases above the theoretical value computed from the amount of moisture which remains infected. This can be accounted for only by a diffusion of acid from the infected droplets to those free from bacteria. This diffusion is probable because the distance between the moisture droplets averages not more than five microns and diffusion of salt into unsalted butter has been established. Overworked butter shows less diffusion.

4. To prevent deterioration of butter by bacteria it seems advisable to churn pasteurized sweet cream with as few bacteria as possible and work it as much as possible without making it salty. Experiments by Guthrie of Cornell University (unpublished) demonstrated that of 10 churnings in 1926 and four churn-

ings in 1927 the highly worked butter scored higher after five months of cold storage than the butter which was worked but very little. However, the distinctly overworked butter scored no higher than the butter with very little working. This last effect might be due to air worked into the butter.

5. Washing decreases acid formation much more than would be expected from the fairly large amount of protein and lactose left in washed butter. This result can be accounted for by the assumption that washing does not change the concentration of the smaller droplets but dilutes the larger drops to almost pure water. As the large droplets are infected, while most of the smaller ones contain no bacteria, the separation between bacteria and food is much more complete than the chemical analysis of the butter will show.

6. These deductions and computations probably will not hold true for molds which have the ability to force their way from one droplet to the other.

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HEAT COAGULATION OF EVAPORATED MILK AS AFFECTED BY MIXING DIFFERENT GRADES OF RAW MILK*

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Over the receiving platform of evaporated-milk factories there comes daily milk of varying quality and degree of purity. The purpose of this investigation has been to ascertain to what extent the heat coagulation of evaporated milk is affected by mixing a poor milk with a larger batch of good quality milk.

When a milk of low acidity is mixed with one of greater acidity a change in the hydrogen-ion concentration of the resulting mixture is observed. The hydrogen-ion concentration of a single sample is increased by bacterial action during aging. These changes in pH will, in turn, affect the heat stability of the milk. Rogers, Deysher and Evans (2) found no definite relationship between the hydrogen-ion concentration and the coagulation temperature of evaporated milks. Sommer and Hart (3) found that a slight increase in acidity increased the heat stability of some milks, while in others the stability was decreased. They attribute this variation in heat coagulation not directly to the hydrogen-ion concentration but to the effect of acidity upon the salt balance. Benton and Albery (1) found that outside the range pH 6.58 to 6.65, changes in pH produced a marked effect on heat stability. They ascribed the variations in stability observed within this range as due chiefly to changes in salt balance.

EXPERIMENTAL

Skim milks of low heat stability were mixed with those of greater heat resisting capacities. The samples were mixed cold, forewarmed to 95°C. for ten minutes, evaporated under vacuum

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TABLE 1

Effect of adding poor milk to good milk on the heat stability of evaporated milk

LOT NUMBER	POOR MILK IN MIXTURE	ALCOHOL TEST	pH	TIME OF COAGULATION AT 120°C.
	per cent			*minutes*
Series 1. Showing an initial increase, then a decrease in heat stability after the addition of poor milk				
127	0	−	6.55	21
	8	−	6.55	26
	16	−	6.53	29
	100	+	6.35	0
201	0	−	6.64	12
	16	−	6.58	22
	32	−	6.53	25
	48	−	6.49	20
	100	±	6.47	3
203	0	−	6.63	21
	6	−	6.56	33
	12	−	6.54	33
	24	−	6.47	18
	36	−	6.42	3
	100	+	6.18	−0
207	0	−	6.54	24
	6	−	6.52	34
	12	−	6.50	38
	24	−	6.48	20
	36	±	6.43	14
	100	+	6.34	−0
403	0	−	6.62	16
	8	−	6.59	23
	16	−	6.57	27
	28	−	6.46	8
	44	±	6.40	4
	68	+	6.30	−0
Series 2. Showing a decrease in heat stability after the addition of poor milk				
324	0	−	6.64	19*
	6	−	6.62	15*
	12	−	6.59	17*
	24	−	6.51	10*
	40	±	6.46	7*
	60	+	6.40	5*

* Coagulation at 115°C.

TABLE 1—*Continued*

LOT NUMBER	POOR MILK IN MIXTURE	ALCOHOL TEST	pH	TIME OF COAGULATION AT 120°C.
Series 2.—*Continued*				
	per cent			*minutes*
209	0	−	6.53	35
	6	−	6.50	28
	12	−	6.49	18
	24	±	6.48	7
	36	+	6.43	6
	100	+	6.25	−0
224	0	−	6.55	27
	12	−	6.55	27
	24	−	6.54	28
	48	−	6.51	25
	72	−	6.49	20
	100	−	6.46	19
223	0	−	6.64	27
	8	−	6.63	23
	16	−	6.61	21
	100	+	6.37	−0

to 18 per cent solids-not-fat, and sterilized at 120°C. in tins until coagulation occurred. Samples were taken before forewarming, on which the pH and the alcohol test were determined.

The good quality milk used was separated skim milk, usually fresh on the morning of the experiment. The poor milk was separated skim, allowed to stand either at room temperature or in ice box until it was alcohol positive or nearly sour. Milk was considered alcohol + when a precipitate occurred with equal parts of milk and 74 per cent alcohol. The ± point was taken as + to 74 per cent and − to 68 per cent alcohol. When − to 68 per cent alcohol the milk was considered alcohol negative. The poor milk added to that of good quality is calculated as a given percentage by weight of the total mixture when made up to the total with the good sample before concentration.

Milks of two different types were found, namely, those whose stability was first increased by addition of poor milk, and those whose stability was decreased under the same conditions. The

milks used were from the United States Experiment Farm at Beltsville, Maryland, and from two local dairies. The milk from each source was consistent in its behavior from day to day.

In table 1, series 1 are shown some data from the experiments on milk from the Beltsville farm which could always be improved during this work by addition of poor milk. Series 2 of the table contains data obtained from the milks of the two local dairies

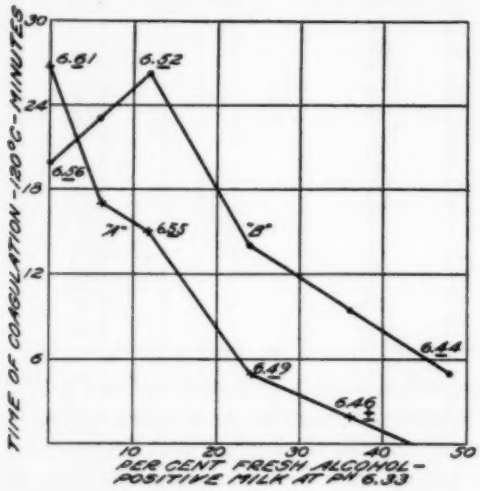

FIG. 1. SHOWING THE EFFECT OF SMALL ADDITIONS OF FRESH ALCOHOL-POSITIVE MILK UPON THE HEAT COAGULATION OF TWO DIFFERENT TYPES OF MILK

The pH and alcohol reactions are indicated

which decreased in stability upon addition of poor milk. Additions of poor milk in any proportion to this type of product were always detrimental in their influence on the heat coagulation of the resulting mixture.

If these milks were not at their optimum pH for maximum heat resistance, then addition of lactic acid should have an effect similar to that of high acid milk developed by bacterial action. Benton and Albery (1) were able to increase greatly the stability of certain samples of fresh milk by addition of various amounts

of lactic acid. Similarly additions of lactic acid to the two types of milks shown in table 1 affected their heat stabilities in the same way as did mixing with aged milk.

It has often been observed that a few cows can be found in many herds whose milk is either continually or occasionally alcohol positive in reaction at the time of milking. The effect of additions of such fresh alcohol-positive milk from three cows habitually giving this type of milk, to fresh normal milks, is

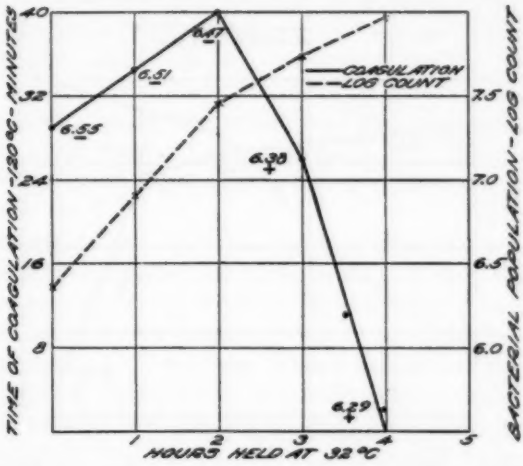

FIG. 2. SHOWING A MILK THE HEAT STABILITY OF WHICH IS FIRST INCREASED THEN DECREASED BY AGING

The pH and alcohol reactions are indicated

shown in figure 1. The pH and the reaction to the alcohol test are shown on the figure for each milk.

Curve A is milk from one of the local dairies, while curve B is for milk from the Beltsville farm. The stability of these samples when mixed with varying percentages of the same fresh, alcohol positive milk is similar to their heat stability when mixed with milks whose quality has been lowered by aging and by increase in acidity. The trend of curve A is typical of the behavior

of the milk in series 2 of table 1. Curve B shows the manner in which the milk reported in Series 1 would plot graphically.

Mixing a poor quality milk with one of better quality should have the same effect upon heat stability as aging a milk of high stability in place of adding poor milk. The pH of the resulting product is changed in both cases by bacterial action.

Samples of milk were aged at 30° to 34°C. until they became alcohol positive.[1] At various intervals samples were taken for

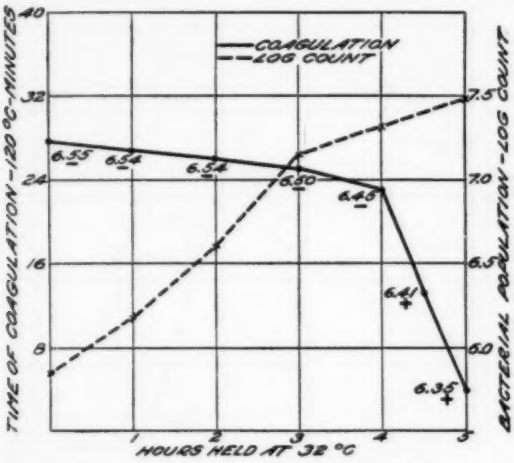

FIG. 3. SHOWING A MILK, THE HEAT STABILITY OF WHICH IS DECREASED BY AGING

The pH and alcohol reactions are indicated

evaporation and coagulation and for bacterial plating and determination of pH. No consistent relationship was observed between the pH changes or the coagulation time and the type of bacterial growth. Colonies were isolated but no outstanding change could be considered a result of the action of an individual organism.

In figure 2 are plotted the data obtained for one of the milks

[1] Data on aged milk is from unpublished work of Anne G. Benton and the author.

which was first increased then decreased in heat stability during aging. The bacterial population has been expressed as the log count of the colonies found.

Figure 3 shows the results for one of the milks, the heat stability of which was decreased by aging.

DISCUSSION

The result of adding small percentages of poor milk to a milk of good quality can be expected either to increase or decrease the initial heat stability of the good sample. No method is available by which the result of such mixing may be foretold. The conclusions of Benton and Albery that each sample of milk, whether mixed or from a single cow, presents a separate colloidal system, appears to apply here. There apparently is an optimum pH for each system, at which optimum the greatest heat stability is obtained.

The nature of the results is the same, whether good and poor milks are mixed, or whether a single milk is aged and the pH developed by bacteria. In both cases the milk was originally at the pH suitable for its greatest stability or it attained that pH during treatment. It has been noticed during this investigation that the periods during which the milk was not normally at its maximum stability were often a week or longer in length.

It is probable that mixed herd milk is usually at its optimum pH originally but that there are sometimes short periods of time when the milk after secretion is not balanced correctly to give it maximum heat stability. At these times adjustment of the pH would be beneficial.

Aging or incubation of the product was always accompanied by an increase in bacterial growth as well as a decrease in pH.

The stability always changed with an increase or decrease in pH.

SUMMARY

When small amounts of poor milk were mixed with larger batches of good quality milk an evaporated product of uncertain heat stability was produced. The change in heat stability which

was observed as a result of mixing quality, of adding lactic acid, or of aging the milk is ascribed to the accompanying change in pH. It is again suggested that for every milk there is an optimum pH, the attainment of which will result in maximum heat stability for the system involved. Identical results in terms of heat coagulation may be obtained by mixing milks of different quality or by aging a single sample.

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THE RELATION OF SOYBEAN HAY AND GROUND SOYBEANS TO FLAVOR AND COMPOSITION OF MILK AND BUTTER*

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Several reports which reached the Department of Dairy Husbandry during the past year stated that rations containing soybean hay or ground soybeans caused undesirable flavors in milk, cream, and butter; these products being affected to such an extent that they were not marketable or that their value was lowered.

Previous investigations (1), (2) have shown that the feeding of soybeans, soybean oil meal, and soybean oil cause the body of butter to be noticeably soft, but so far as the authors can learn no investigations have been reported in which harmful flavors resulted from rations of this character. In fact, Lindsey et al. (2) report that soybean oil meal imparted no particular flavor to butter.

Experiments were therefore undertaken with the object of determining the effect of rations containing soybean hay and ground soybeans upon the flavor and composition of milk, cream, and butter.

Twenty-four cows each producing daily 15 to 30 pounds of milk were divided into three groups. Division was made on the basis of breed, age, live weight, days from last calving, stage of gestation, and milk yield. The average daily production of each group at the beginning of the experiment was approximately 20 pounds per cow. On account of advancing lactation in one case and indigestion in another, it was necessary to omit the records of two cows during the last week of the experiment. The experimental periods were each one week in length.

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EXPERIMENTAL RATIONS AND PROCEDURE AT BARNS

During the first week, all groups were fed the control ration consisting of red clover hay and "herd mixture" (300 pounds ground corn, 400 pounds ground oats, 200 pounds wheat bran, 100 pounds linseed oil meal, 15 pounds bonemeal, 15 pounds salt). Group A was continued on this ration throughout the experiment.

During periods 2, 3, 4, and 5, group B was fed soybean hay and a concentrate mixture. The soybean hay fed during periods 2, 3, and 4 was of high grade, having been cut when the beans were in the early stages of development. It had a high green color and was very palatable. The leaves formed 45 per cent of the weight of the hay and the pods and beans 17 per cent. Seven per cent of weeds, mostly foxtail, was present.

It seemed desirable to learn the effect of poor quality soybean hay also. Accordingly, during period 5, some badly weathered soybean hay having many large, moldy beans and pods was fed. The leaves formed 21 per cent, the beans 21 per cent, and the pods 22 per cent of the weight of the hay. Four of the cows refused this hay, so their records are omitted from this period. The grain mixture fed group B during period 2 was the herd mixture. This was replaced during periods 3, 4 and 5 by grain mixtures containing 10, 15, and 20 per cent of ground soybeans, the same mixtures as fed to group C during periods 2, 3, and 4.

Group C was fed the same kind of hay as group A. During periods 2, 3, 4, and 5, the grain mixtures consisted of ground corn, ground oats, and wheat bran, together with enough ground soybeans to form approximately 10, 15, 20, and 25 per cent of the mixtures in the respective periods. Bonemeal and salt were added at the rate of about 1.5 per cent each.

During period 6, both groups B and C were returned to the control ration of clover hay and herd mixture. No silage was fed during the experiment. All the experimental periods were consecutive, changes in rations being made abruptly.

A condensed summary of the amounts of feed consumed, together with the production records of the different groups, are shown in table 1. Hay consumption was large, ranging from 20

to 25 pounds per cow daily in most of the periods. Concentrates were fed at the rate of about 1 to 2½ pounds of milk, the maximum

TABLE 1

Production and feed records of cows during study of the effect of soybeans on the flavor and composition of milk and butter

Results expressed in terms of weekly averages per cow

WEEK	RATION	MILK	FAT	FAT	F. C. M.†	LIVE WEIGHT	FEED EATEN	
							Hay	Grain
	Group A							
			per cent	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1	Clover hay—herd mixture	127.9	3.90	4.99	126.01	1,134	140.3	58.9
2	Clover hay—herd mixture	121.5	3.89	4.72	119.40	1,127	150.7	55.4
3	Clover hay—herd mixture	118.3	3.88	4.59	116.17	1,132	157.4	49.0
4	Clover hay—herd mixture	103.5	3.88	4.02	101.70	1,131	153.2	46.4
5‡	Clover hay—herd mixture	93.5	4.07	3.80	94.40	1,116	161.7	44.0
	Group B							
1	Clover hay—herd mixture	140.2	3.85	5.40	137.08	1,192	137.0	60.0
2	Soybean hay—herd mixture	142.7	3.84	5.48	139.28	1,163	124.0	60.9
3	Soybean hay—mixture no. 1*	134.3	3.87	5.20	131.72	1,155	148.0	55.7
4	Soybean hay—mixture " 2*	133.1	3.95	5.26	132.14	1,158	156.1	56.0
5§	Soybean hay—mixture " "*	144.6	3.77	5.45	139.59	1,072	159.5	61.3
6‡	Clover hay—herd mixture	126.2	3.78	4.78	122.18	1,113	154.7	56.0
	Group C							
1	Clover hay—herd mixture	141.3	4.16	5.87	144.57	1,152	148.2	60.0
2	Clover hay—mixture no. 1*	143.4	4.01	5.75	143.61	1,135	163.7	60.1
3	Clover hay—mixture " 2*	144.2	4.12	5.95	146.93	1,145	166.4	61.3
4	Clover hay—mixture " 3*	138.5	4.14	5.74	141.50	1,136	156.3	57.4
5	Clover hay—mixture " 4*	136.4	4.03	5.50	137.06	1,158	172.5	56.9
6	Clover hay—herd mixture	135.0	4.02	5.43	135.45	1,152	172.0	56.0

* Mixture no. 1 contained 10 per cent ground soybeans; no. 2, 15 per cent; no. 3, 20 per cent; no. 4, 25 per cent.

† F. C. M. (fat corrected milk) equals .4 × milk + 15 × fat. (Ill. Agr. Exp. Sta. Bul. 245).

‡ Seven cows only.

§ Four cows only.

amount of soybeans fed being about 2½ pounds per head daily. Barn records of milk yield and weekly composite samples taken

from each milking served as a basis for the computation of production records. The samples were tested for butterfat by the Babcock method. The butterfat tests shown in this table thus differ slightly from the averages of the butterfat contents of the mixed milk as determined by the Mojonnier method.

Care was taken at the barn to keep the milk as clean and free from odors as possible. Cans and strainers were provided for the milk of each group. These cans were distinguished by covering the shoulders with paint of different colors, red representing the control ration, yellow the soybean hay ration, and blue the ground soybeans ration. The same color scheme was followed on the feed sheets, milk records, grain mixtures, and cards over the mangers.

The milk was taken daily to the Dairy Manufactures Building, where tests were carried out to determine the effect of the rations upon the flavor and composition of milk and butter.

PROCEDURE AT DAIRY MANUFACTURES BUILDING

The milk was first clarified. (During the first week, the milk was filtered through ordinary milk strainers, but this procedure was found impractical due to the time involved.) Samples of the raw milk were taken for cream volume studies and judging. The milk was then pasteurized by heating to 142°F. and holding for thirty minutes at that temperature. A 50-gallon starter can type of vat was used for this purpose. A portion of the milk was then pumped over a surface cooler. The remaining milk was pumped into a power separator and separated at about 120°F. The skim milk and cream were then pumped over the surface cooler and sampled.

Portions of the raw, and the cooled pasteurized whole milk were placed in 100 cc. graduates in a 40°F. refrigerated room for the purpose of cream volume measurement. These tests were run in duplicate and were read approximately twenty-four hours after setting.

The raw milk was tested daily for fat and total solids using the Mojonnier Milk Solids Tester. Acidity determinations were also made on the raw milk using N/10 NaOH and phenolpthalein as an indicator.

The raw and pasteurized whole milk, the pasteurized skim milk and cream were judged daily for odor and flavor.

The pasteurized cream was held at 40°F. in 10-gallon milk cans and at the end of seven days the accumulated cream was churned. The cream was standardized to 35 per cent fat with fresh pasteurized skim milk and 10 per cent of starter added. A Cherry Junior Churn (No. 2B) with a capacity of about 30 gallons of cream was used. The churning procedure followed was the standard procedure for the creamery.

The butter was scored one day after being made and again about one week later. From seven to eight individuals sampled the butter. The score on the butter was placed by H. A. Ruehe and P. H. Tracy of the Dairy Manufactures Division.

Each lot of butter was analyzed for fat, moisture, salt, and curd using the modified Kohmann method. In addition the Iodine Number (Hanus) and the Refractive Index of each lot was determined. An attempt was made to determine the melting point of the butter but reliable checks could not be obtained.

SOYBEANS HAD NO EFFECT ON MILK FLAVOR

Samples of the milk before and after it was pasteurized as well as the cream and skim milk resulting from separating the pasteurized milk were judged daily for flavor. In no case was it possible to detect any flavor that might be attributed to either the ground soybeans or soybean hay. The flavor of the milk varied. In some cases the control lot was the best. In other cases the milk from the soybean groups was the better. In several instances the three lots were equally good. In practically all cases where a defect in flavor occurred it could be attributed to factors other than feed such as a cowy flavor or barn odors. It was noticed that the milk from group C, during the interval this group was receiving the maximum amount of ground soybeans, had a flavor different from the other groups, but it was not an objectionable flavor. However, this flavor which was rather noticeable at the beginning of the period was hardly detectable in the milk received during the last two days of the week. On the whole, the milk of groups B and C was fully as good, if not better, in flavor as that of group A.

COMPOSITION OF MILK AFFECTED SLIGHTLY

The data are not comprehensive enough to draw definite conclusions as to the effect of either ground soybeans or soybean hay upon the fat content of the milk. The data, given in table 2, indicate that after groups B and C had been returned to the control ration in period 6, that the omission of soybeans from the ration seemed to cause a slight lowering of the fat content of the milk.

TABLE 2

*Composition of milk as affected by soybean rations**

WEEK	FAT IN MILK OF GROUP			SKIM MILK SOLIDS OF MILK OF GROUP			ACIDITY OF MILK OF GROUP			CREAM VOLUME OF MILK OF GROUP					
										A		B		C	
	A	B	C	A	B	C	A	B	C	Raw	Pasteurized	Raw	Pasteurized	Raw	Pasteurized
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
1	4.09			9.06			0.152			14.6	11.6				
2	4.12	3.99	4.22	8.82	8.83	9.17	0.152	0.155	0.152	13.6	11.9	12.3	11.3	15.3	13.9
3	3.98	4.07	4.36	8.91	8.88	9.06	0.154	0.153	0.153	12.9	11.1	12.9	11.4	15.7	14.7
4	4.05	4.09	4.25	8.88	8.92	9.09	0.153	0.153	0.154	12.9	11.8	12.1	11.6	14.9	14.4
5	4.27	4.34	4.29	9.01	8.93	8.97	0.153	0.151	0.154	14.4	12.1	13.0	11.9	15.4	14.2
6		4.08	4.08		8.95	9.07						14.1		16.4	

* Computed from weighted averages for entire period.

The soybean rations had no effect upon the acid content of the milk as measured by titration with N/10 NaOH.

DIFFERENCES FOUND IN QUALITY OF BUTTER

None of those sampling the butter were able to detect any flavor defect that could be attributed to the soybeans. In several instances the flavor of the butter from the groups fed soybeans was better than that of the control. All of the butter produced while clover hay was being fed, with the exception of period 2, had what was designated as an unclean flavor. In only one lot of butter, that from the soybean hay ration of period

4, was an unclean flavor found, and it was not so pronounced as that of the butter from the control ration during the same period.

There was a noticeable effect of soybeans upon the body of the butter. The butter from the soybean groups seemed to have

TABLE 3

Composition and score of butter as affected by soybean rations

WEEK	FAT IN BUTTER	IODINE NUMBER	REFRACTIVE INDEX	SCORE OF FLAVOR		SCORE OF BODY		TOTAL SCORE	
				(1)	(2)	(1)	(2)	(1)	(2)
Group A. Control ration									
	per cent								
1	83.9	40.05	1.4547	36.5	36.0	24.5	24.5	91.0	91.0
2	82.6	40.62	1.4547	37.0	37.5	24.5	24.5	91.5	92.0
3	83.1	40.50	1.4548	36.0	36.5	24.0	24.0	90.0	90.5
4	82.3	41.66	1.4548	36.0	36.5	25.0	25.0	91.0	91.5
5	82.0	40.57	1.4548	35.0	33.0	24.0	24.0	89.0	87.0
Group B. Soybean hay ration									
2	82.9	40.02	1.4547	37.0	37.5	24.0	24.0	91.0	91.5
3	83.6	39.46	1.4547	37.5	37.5	24.0	24.0	91.5	91.5
4	83.1	40.09	1.4548	37.0	36.5	24.0	24.0	91.0	90.5
5	83.3	43.13	1.4554	35.0	35.0	23.0	23.0	88.0	88.0
6*	82.5	44.02	1.4554	36.0	35.0	24.3	24.3	90.3	89.3
Group C. Ground soybeans ration									
2	82.6	39.64	1.4546	37.0	37.5	23.5	23.5	90.5	91.0
3	80.1	40.55	1.4547	37.5	37.5	23.5	23.0	91.0	90.5
4	82.2	41.18	1.4547	37.5	37.0	24.0	23.5	91.5	90.5
5	82.5	41.80	1.4550	35.5	35.5	23.0	22.5	88.5	88.0
6*	83.1	40.90	1.4548	36.0	35.0	24.5	24.5	90.5	89.5

* Control ration fed.

(1) Scored day after churning

(2) Scored one week after churning.

more of a gummy consistency than the other (this butter was judged at a temperature of about 40°F.). The ground soybeans was more of a factor in contributing to this condition than the soybean hay. This defect was not serious, although as shown in table 3, the score of the body of the butter from the soybean

rations was usually from one-half to one and one-half points lower than that of the butter from the control ration.

The results obtained in the butter constants determinations are not conclusive. It was impossible to obtain satisfactory melting points. The refractive index values were fairly uniform though there was a tendency for the iodine number to increase with the increased soybean content of the ration. This increase was more consistent in the case of the ground soybeans ration than in the case of the soybean hay ration.

CREAM VOLUME OF MILK IS HIGHLY VARIABLE

An interesting phase of this study was that of cream volume. There was a marked tendency for volume of cream in the group C milk samples to exceed that in the milk from the other groups. This relationship seemed to hold true even when the fat content of the group C milk was equal to or less than that of the other milk.

Determinations of cream volume of the milk of the individual cows of groups B and C were made during period 6, after both groups had been returned to the control ration. The results indicate that the variation in cream volume was not due to the effect of feed. The explanation for the higher cream volume of the milk of group C seems to be that this group included individual cows yielding milk having a greater cream volume than the milk of the cows of group B.

SUMMARY

Three groups of eight cows each were fed during six consecutive weekly periods on rations consisting of either red clover hay or soybean hay, and a concentrate mixture with or without ground soybeans. The ground soybeans, when fed, formed from 10 to 25 per cent of the mixtures.

The milk produced was used in studies of flavor and composition, and butter was made from the cream.

Neither high quality, nor poor quality, moldy soybean hay was found to have any effect upon the flavor of the milk (raw or

pasteurized), skim milk, cream, or butter. Ground soybeans were likewise without effect on flavor. The fat content of the milk was affected but slightly, and the acidity was not changed appreciably. Marked differences in cream volume were noted but these seemed to be associated with individual cows.

The most pronounced effects of the rations were upon the body of the butter. Ground soybeans caused the body to be gummy and the condition became worse when the proportion of soybeans in the ration was increased. Soybean hay had a similar effect but to a less degree. There was a tendency for the iodine number to increase slightly with the larger amounts of soybeans in the ration.

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COTTONSEED MEAL AS A FEED FOR DAIRY CALVES*

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Cottonseed meal is seldom recommended as a feed for calves, and only limited amounts are recommended for lactating dairy cows. This is due to a common opinion that cottonseed meal contains a poisonous principle which produces cottonseed meal injury (1). During the last five years, 20 Holstein heifers were grown from six to twenty-four months of age at Michigan State College on a grain ration consisting of cottonseed meal as the principal source of protein. The animals also received an ample supply of timothy hay and corn silage. No physical defects were produced although 2 pounds of cottonseed meal were fed to these animals daily throughout this period. The present paper is a preliminary report covering the effects of cottonseed meal feeding on the health of growing dairy heifers. The effects on reproduction and lactation will be reported later.

REVIEW OF LITERATURE

Although Nevens (2), Aberhalden and Rostoski (3), Richardson and Green (4), Mendel (5), Osborne and Mendel (6), and McCollum and Simmonds (7) have demonstrated the high biological value of cottonseed protein, several investigators have reported injurious effects from feeding cottonseed meal to cattle. Dinwiddie and Short (8) state the symptoms of cottonseed meal poisoning in cattle are recognized by a reeling unsteady gait, which appears to be due in part to muscular incoördination and, in part, in the later stages, to partial or complete blindness. These workers produced cottonseed meal injury in two Jersey steers. One animal which received 1 pound of cottonseed meal daily for each 100 pounds of live weight, with cottonseed hulls for roughage

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showed the symptoms of cottonseed meal poisoning at the end of 70 days. This animal was anemic, having an erythrocyte count of only 3,400,000 and a hemoglobin of 50 per cent. The other steer did not develop the symptoms of cottonseed meal injury until after 116 days of cottonseed meal feeding. Jones, Lush, and Jones (9) found a lessening of appetite with a consequent decrease in the rate of gains as the preliminary symptoms of cottonseed meal injury in fattening steers.

In order to determine the effect of cottonseed meal upon growth and reproduction of cows, Combs and Curtis (10) placed five heifers on each of the following rations:

Ration 1. Cottonseed meal (1 pound daily per 100 pounds live weight). Cottonseed hulls ad libitum.

Ration 2. Cottonseed meal ½, crushed corn ½ (1 pound daily per 100 pounds live weight). Cottonseed hulls ad libitum.

Ration 3. Cottonseed meal (1 pound daily per 100 pounds live weight). Cottonseed hulls ½ and corn silage ½, ad libitum.

Ration 4. Cottonseed meal (1 pound daily per 100 pounds live weight), copper sulphate solution. Cottonseed hulls ad libitum.

Ration 5. Cottonseed meal (1 pound daily per 100 pounds live weight). Cottonseed hulls ½ and beet pulp ½, ad libitum.

Some of the heifers became weak, had convulsions, and became blind. All of the heifers in lot 1 died, as did some of the animals in the other lots. Those that lived became weak and were becoming blind so they were removed from the experiment.

McNutt (11) reported that a bull calf fed on skim milk and cottonseed meal died after 71 days on this ration. One-fourth pound of cottonseed meal was fed per day at first, but this amount was gradually increased to 1½ pounds per day. In another experiment, McNutt fed one group of heifers a basal ration of corn, oats, and wheat bran, equal parts by weight, and hay, and corn silage. Another group were fed cottonseed meal, corn, oats, hay, and silage. The four younger heifers in the latter group either failed to gain or lost considerable weight during the first two months on this ration. They also appeared very unthrifty. When placed on the basal ration they made very satisfactory

gains. According to Kennedy and Marshall (12), three head of cattle in a lot of 20 died after receiving a ration consisting of 25 pounds of corn and cob meal, 2½ pounds cottonseed meal, and wheat straw for roughage for 42 days. The rest of the animals became blind.

Many theories have been advanced attempting to explain the cause of cottonseed meal injury. Only a few are enumerated in this report. Crawford (13), working with rabbits concluded that the chief ptomaine principle in cottonseed meal was a salt of pyrophosphoric acid. However, Withers and Ray (14) were unable to substantiate Crawford's conclusions. Forbes, Beegle, and Mensching (15) found the excess of acid over base forming elements in 100 grams of cottonseed meal was equal to 7.7 cc. of normal acid. Wells and Ewing (16) reported that the excess of acid over base forming elements was 8.21 cc. of normal acid. They then thought that cottonseed meal injury might be due to an acidosis. Later, in an extensive investigation dealing with swine, they were unable to produce evidence to support this hypothesis. Rommel and Vedder (17) found that symptoms of injury produced when pigs were fed a ration of nine parts steamed polished rice and one part tankage were quite similar to the symptoms produced in other pigs which were fed a ration of two parts corn and one part cottonseed meal. These men then concluded that beri-beri and cottonseed meal injury were analogous diseases. However, it is unlikely that cottonseed meal injury in cattle is analogous to beri-beri since Bechdel, Eckles, and Palmer (18) showed that the vitamin B requirement of calves is either extremely low or that it is synthetized in the animal body. McGowan and Crichton (19) stated that the hemoglobin of pigs suffering from cottonseed meal injury was sometimes as low as 20 per cent although it was generally about 40 per cent. This condition was relieved by feeding 40 grams of ferric oxide daily to the mothers of the suckling pigs. The investigators concluded that the principal factor causing cottonseed meal injury was a lack of sufficient iron in the ration. Withers and Carruth (20) reduced the injury in swine which were fed cottonseed meal and corn by adding either copperas or ferric chloride to the ration.

Withers and Brewster (21) found that certain iron salts tended to alleviate the toxicity produced in rabbits by the feeding of cottonseed meal.

Withers and Carruth (22) were the first investigators to attribute the injury produced by cottonseed meal to gossypol. They have shown (23) that gossypol is toxic when fed to rats, rabbits, guinea pigs, or swine.

The theory that gossypol is responsible for cottonseed meal injury was further substantiated by Schwartze and Alsberg (24) who found that diets containing 0.225 per cent of gossypol killed rats within three days.

Sherwood (25) found that gossypol in 14 samples of cottonseed meal, prepared from kernels cooked in open kettles, varied from 0.021 to 0.150 per cent and the d-gossypol content from 0.544 to 0.963 per cent. Twenty-two samples of meal from mills using the continuous cooker process varied from 0.007 to 0.228 per cent of gossypol and from 0.633 to 1.076 per cent of d-gossypol. Four samples of meal made by the expeller process varied from 0.02 to 0.102 per cent of gossypol and from 0.335 to 0.505 per cent of d-gossypol.

Menaul (26) produced death in a four pound rabbit in about four minutes by injecting 0.1 gram of gossypol into the marginal vein of the ear. A half gram administered orally to another rabbit produced no abnormal symptoms. Another rabbit injected intraperitoneally with 0.5 gram died on the fourth day. Continued feeding of 0.1 gram of gossypol per day to each of four rabbits caused intestinal inflammation resulting in death in about 14 days. He found the gossypol tended to inhibit the liberation of oxygen from oxyhemoglobin. Only 46.8 per cent as much oxygen was liberated from 2 cc. of erythrocytes in 1 cc. of physiological salt solution having 0.02 gram of gossypol, as from the controls.

Withers and Carruth (23) maintain that, when cottonseeds are heated in the presence of water, gossypol is converted to less soluble d-gossypol, which is also less toxic. They account for the variation in toxicity by the variation of gossypol amd moisture in the cottonseed from which the meal is made, and by the variation

in heating during the manufacturing process. The works of Sherwood (25), Dowell and Menaul (27), Gallup (28), Edgerton and Morris (29) tend to support this view. Osborne and Mendel (30) found cottonseed meal non toxic, but cottonseed kernels that contained about 0.6 per cent of gossypol were toxic to rats. Nevens (31) and Richardson and Green (4) also showed that cottonseed meal and cottonseed flour are not toxic to rats. There is no evidence that the injury produced in cattle which are fed cottonseed meal is due to gossypol.

Several investigators have reported that the injurious effects of cottonseed meal are at least partially neutralized by feeding other feeds with the cottonseed meal.

Wright (32) fed 2000 head of cattle a daily ration of 8 pounds of cottonseed meal and 25 pounds of cottonseed hulls, with a feed of hay once a week throughout the fattening period. These cattle (mostly four to six-year-old steers) made an average gain of 75 pounds each per month. The only abnormality observed was an occasional diarrhea, which was relieved by feeding hay for a few days. Stanley (33) concluded from results secured in steer feeding work that, if silage replaced cottonseed hulls as roughage, cottonseed meal might be fed for a longer time without ill effects. Burtis (34) concluded that some other grain and hay should be fed with cottonseed meal to fattening steers. Michels (35) reports, "Our feeding trials have well established the fact that cottonseed meal can be fed with much greater safety in conjunction with silage than with dry roughage." Michels did not state whether the dry roughage was hay, straw, or cottonseed hulls.

The North Carolina Station (36) reported that a herd of 20 cows fed heavily on cottonseed meal were apparently normal when kept in a four acre pasture. When this herd was moved to a dry lot, abortion, dead or underweight calves, and living blind calves were obtained. The cows also gradually lost weight, became stiff, and had edema. In some cases these animals had severe fits from which they ultimately died. Other cows had fits occasionally and became partially blind and some permanently blind. These results suggested nutritive deficiencies in rations consisting of cottonseed meal and hulls. Such rations supplemented by vitamins A and B and calcium gave good results.

EXPERIMENTAL

Part I

The similarity of the symptoms of cottonseed meal injury in cattle to those produced when concentrates alone were fed suggested the possibility that both conditions might be a result of the same deficiency. In order to study this relationship, two lots of two calves each were used. The animals in lot 1, C 43 and C 47, received cottonseed meal as the principal source of protein while those in lot 2, C 44 and C 45, received corn gluten meal and corn distillers grain. Both groups received corn and oats and wheat

TABLE 1

Feed record of calves fed cottonseed meal with wheat straw as a roughage

Lot 1—Part I

30-DAY PERIODS	C 43					C 47				
	Skim milk	Cotton-seed meal	Yellow corn	Oats	Wheat straw	Skim milk	Cotton-seed meal	Yellow corn	Oats	Wheat straw
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1	360	66	46	46	Ad. lib.	362	43	46	46	Ad. lib.
2	308	74	70	70	Ad. lib.	420	60	60	60	Ad. lib.
3	785	160	29	29	Ad. lib.	420	91	45	45	Ad. lib.
4	15	168	29	29	Ad. lib.		112	45	45	Ad. lib.
5		180	30	30	Ad. lib.		52	27	27	48
6		180	33	33	Ad. lib.		82	43	43	57
7		153	45	45	Ad. lib.		14	8	8	9†
8		159	45	45	78					
9		60	30	30	54*					

* Twenty days.

† Eight days.

straw ad libitum. Salt was placed before the animals at all times. Skim milk was fed to 180 days of age. The animals used were four bull calves, three Holsteins and one Jersey, which had received an adequate ration up to the time they were placed on experiment which was about 90 days of age. The amount of feed consumed by 30 days periods is recorded in table 1. Growth of the calves is found in figures 1 and 2.

Figures 1 and 2 show that the gain in body weight of all four

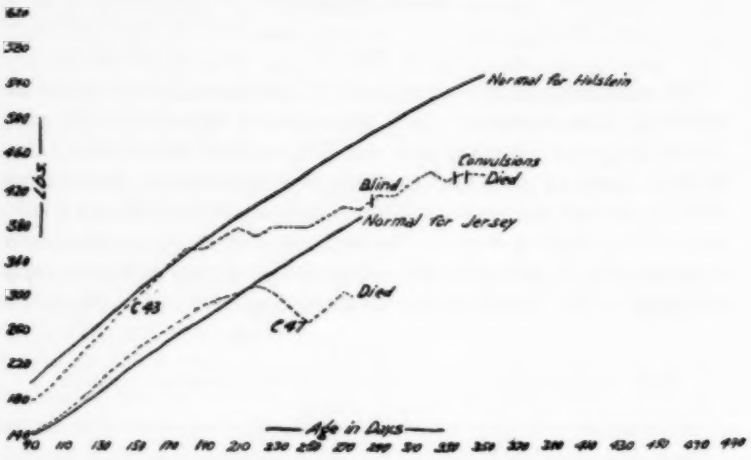

FIG. 1. GROWTH IN WEIGHT OF CALVES RECEIVING COTTONSEED MEAL AS THE CHIEF SOURCE OF PROTEIN WITH WHEAT STRAW AS A ROUGHAGE

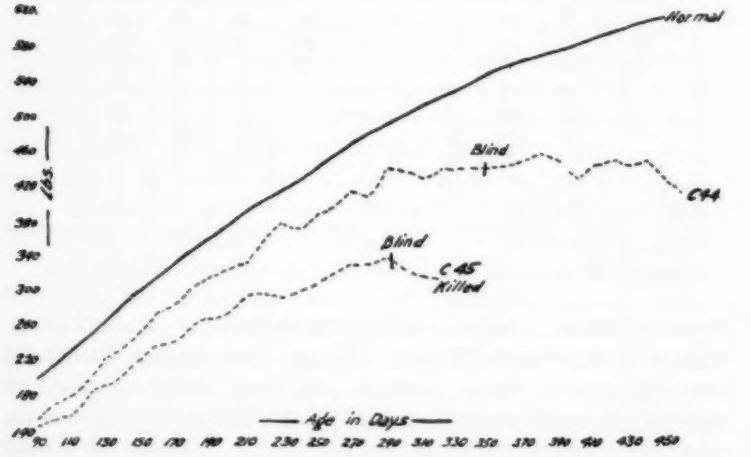

FIG. 2. GROWTH IN WEIGHT OF CALVES RECEIVING CORN GLUTEN MEAL AND CORN DISTILLERS GRAIN AS THE PRINCIPAL SOURCE OF PROTEIN WITH WHEAT STRAW AS A ROUGHAGE

animals was fairly good for a time. The rate of gain was more rapid and the ensuing decline greater in case of the 2 animals receiving cottonseed meal than where corn gluten and corn distillers were fed. This was probably due to the greater palatability of the cottonseed meal which caused an increased intake of concentrates by calves C 43 and C 47. Stiffness and swelling around the hocks were observed in all four animals. C 43 had a convulsion that lasted about ten minutes on the 287th day of age. Seven days later another convulsion of short duration was observed. Diarrhea was also manifested from time to time. This animal was found dead on the 350th day of age. It probably died in convulsions. C 47 died at 275 days of age. This animal did not become blind, nor manifest tetany, although the heavy feeding of concentrates injured its health.

Calves C 44 and C 45 became blind on a ration free from cottonseed meal. C 44 became completely blind at 347 days of age and C 45 at 293 days of age. Neither of these calves died from the effects of the ration but their condition was so poor that C 45 was killed and the ration of C 44 changed.

The histopathological study of the glands and organs of calves C 43 and C 45 and C 47 made by Dr. Delez of the Department of Animal Pathology (37) seemed to indicate that the optic nerves, kidneys, and liver are the most seriously and frequently affected organs. Sections of the wall of the rumen, intestines, adrenal, thyroid, pineal, and the thymus failed to show more than very slight, if any, abnormal changes. The testes of C 43 and C 45 were apparently normal, while those of C 47 showed considerable cellular infiltration of the intertubular tissue.

The liver changes consisted of: Considerable congestion which extended some distance below the capsule into the parenchyma. In the region underneath the capsule, a number of pyknotic cells were seen. There was considerable degenerative change of the parenchyma cells. There was a cellular infiltration of the portal sheaths underneath the capsule, as well as a fibriotic condition around some of the bile ducts.

A section of one of the optic nerves of C 43 showed a degeneration (plate 1) of the myelin sheaths in the center of the nerve.

This degenerative change probably did not extend throughout the entire nerve, since a section from another portion of the same nerve did not reveal this change. A parallel section of the right optic nerve of C 45 (plate 2) showed a constricted portion. Since C 47 was not blind, no portion of the optic nerve was saved for histological study.

Pathological changes in the kidneys are shown in plates 3, 4, 5, and 6. Foci of intertubular proliferation (focal interstitial

TABLE 2

Feed record of calves fed corn gluten meal and corn distillers' grain with wheat straw a roughage

Lot 2—Part I

30-day periods	C 44						C 45					
	Skim milk	Corn gluten	Corn distillers	Yellow corn	Oats	Wheat straw	Skim milk	Corn gluten	Corn distillers	Yellow corn	Oats	Wheat straw
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1	360	17	35	19	19	Ad. lib.	360	6	33	23	23	Ad. lib.
2	360	60	15	30	30	Ad. lib.	360	51	21	27	27	Ad. lib.
3	360	70	5	35	35	Ad. lib.	360	61	10	36	36	Ad. lib.
4		93	0	45	45	Ad. lib.	120	90	0	38	38	Ad. lib.
5		84	13	42	42	66		69	5	38	38	Ad. lib.
6		68	17	34	34	72		68	17	34	34	66
7		77	19	38	38	84		75	18	37	37	78
8		80	20	40	40	90		35	9	17	17	36*
9		80	20	40	40	90						
10		77	19	38	38	87						
11		75	18	37	37	84						
12		80	20	40	40	90						

* Twenty days.

nephritis) were present. Atrophy of the tubular epithelium of the proximal tubules was noted. Those were dilated and contained albumin (chronic parenchymatous nephritis). A few areas along the surface of the cortex showed cellular infiltration (mononuclears and a few lymphocytes, but no polymorphs). The glomerular capsules in the area involved were thickened and there was also a thickening of the renal capsule covering this area. The evidence seems to indicate a chronic interstitial nephritis.

Part II

The results of Part I indicated that the cause of cottonseed meal injury in cattle may be due to too heavy feeding of concentrates in proportion to roughage. In order to determine the effects of feeding cottonseed meal in reasonable amounts with ample roughage of high quality, ten grade Holstein calves were placed on experiment at about 90 days of age. The inheritance of the animals in both lots was similar. Eight of the heifers were sired by the same bull. The dams of all the animals used in this experiment were related.

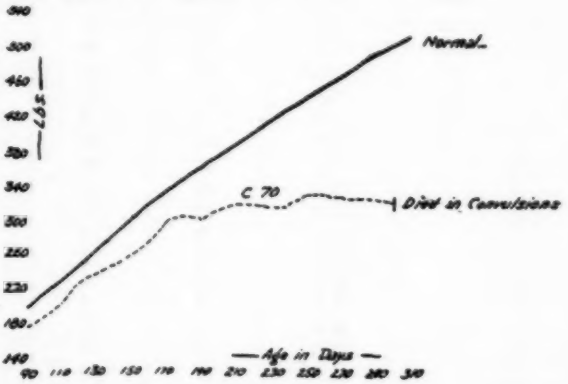

FIG. 3. SHOWING EFFECT OF THE COTTONSEED MEAL USED IN THIS INVESTIGATION ON GROWTH AND HEALTH WHEN FED WITH A ROUGHAGE OF POOR QUALITY

These animals were divided into two lots of five heifers each. Animals in lot 1, G 1, G 3, G 5, G 7, and G 9, received cottonseed meal as the principal source of protein. Those in lot 2, G 2, G 4, G 6, G 8, and G 10, received linseed oil meal as the principal source of protein. The latter lot was used as a check since linseed oil meal is recognized as a safe protein concentrate to use as a supplement for dairy calves.

Rations. Both lots received skim milk until about 150 days of age. They were also fed throughout the experiment all the timothy hay and corn silage which they would clean up. Bone

meal was fed as 2 per cent of the protein concentrate to both lots of heifers. They were allowed free access to salt. Yellow corn was fed in order to equalize total digestible nutrients. The animals in lot 1 received 0.5 pound of cottonseed meal per day until 150 days of age, after which enough cottonseed meal was fed

TABLE 3

Feed record of calf C-70, which was fed principally from the shipment of cottonseed meal used in this investigation

10-DAY PERIODS	SKIM MILK	COTTONSEED MEAL†	CORN	OATS	WHEAT STRAW
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
1*	120	4.6	10	10	3
2	120	11.5	10	10	5
3	120	12.0	10	10	11
4	120	25.0	10	10	9.5
5	120	25.0	9	9	7
6	88	28.0	10	10	11.5
7		35.0	10	10	17.5
8		42.5	10	10	25
9		45.0	10	10	18
10		40.5	9	9	16
11		40.5	9	9	16
12		45.0	10	10	12
13		45.0	7	7	12
14		45.0	9	9	10
15		45.0	10	10	10
16		45.0	10	10	10
17		40.5	9	9	9
18		27.0	6	6	7
19		27.0	6	6	7
20‡		9.0	2	2	4

* Age at beginning of experiment, 96 days.

† Two per cent steamed bone meal and 1 per cent salt were added to the cottonseed meal.

‡ Four days.

to meet the protein requirement according to the Armsby feeding standard. The protein furnished in the roughage and corn was allowed as excess protein. The cottonseed meal used in this experiment during the first nine months of the experiment was from the same lot as that fed C 43 and C 47. The shipment used later was tested biologically by feeding it to calf C 70.

C 70, a Holstein bull calf, was placed on a ration of cottonseed meal, wheat straw, corn, oats, bone meal, and salt at 96 days of age. Skim milk was also fed until 150 days of age. Figure 3 gives the growth of this animal. Food consumption for C 70 is reported in table 3. Death occurred as a result of convulsions at 301 days of age.

Old process linseed oil meal was fed to the animals in lot 2 as the chief source of protein supplement. These heifers were fed

TABLE 4

Growth and feed records of animals fed cottonseed meal or linseed oil meal along with a ration otherwise adequate

NUMBER OF ANIMAL	GROWTH RECORD						FEED RECORD TO MONTHS OF AGE						
	At the beginning	Initial weight	Per cent normal*	Weight at 18 months	Per cent normal*	Average daily gain	Skim milk	Cottonseed meal	Linseed oil meal	Corn	Silage	Timothy	Salt
	days	*lbs.*		*lbs.*		*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
G 1	118	245	99.71	811	118.23	1.34	396	918.3		178	4,373	3,091	16
G 3	90	169	84.5	807	117.64	1.42	723	923.7		242	4,474	3,102	9
G 5	89	146	73.54	732	106.71	1.09	742	909.5		309	3,543	2,919	11
G 7	89	161	81.09	701	102.19	1.00	990	872.0		299	3,516	2,723	23
G 9	94	190	91.99	747	108.9	1.25	960	863.7		288	3,370	2,784	20
G 2	119	300	121.3	922	134.41	1.48	384		1,176.2	19	4,414	3,097	14
G 4	93	203	99.08	831	121.14	1.40	741		1,162.7	168	4,346	3,009	12
G 6	94	198	95.87	779	113.55	1.30	720		1,113.9	136	3,800	3,013	11
G 8	89	165	83.11	763	111.23	1.32	978		1,090.7	188	3,616	2,860	23
G 10	92	199	97.91	832	121.29	1.44	720		1,108.3	140	3,864	2,989	18

* Eckles, C. H. 1920. University Missouri Agr. Exp. Sta. Research Bul. 36.

0.7 pound daily until 150 days of age, after which the intake was gradually raised to three pounds of linseed oil meal per day.

Health. There has been but very little difference in the health of the two lots of heifers thus far. The animals which have received 2.4 pounds of cottonseed meal daily for some time have failed to show swollen joints, stiffness, blindness, or convulsions, which are the symptoms of so called cottonseed meal injury in cattle. Growth in weight often reflects the health of the animal. The gain in body weight of the heifers in lots 1 and 2 is shown in

table 4. The animals in both groups were below normal in weight at the beginning of the experiment with the exception of G 2 in lot 2. Nevertheless, at eighteen months of age all the animals in both groups were above normal. This experiment was not designed, however, to test the relative efficiency of linseed meal and cottonseed meal for growth. The groups were not divided so as to make it possible to measure the relative growth. As a matter of fact, the calves fed on linseed meal were larger and stronger at the start than those fed cottonseed meal.

Hide and Hair. It is common opinion among livestock feeders that the feeding of linseed oil meal to cattle results in a sleeker coat of hair and a more pliable hide than when cottonseed meal is

TABLE 5

Rate of food passage through digestive tract

Lot 1

G 1—AGE 255 DAYS			G 3—AGE 213 DAYS			G 5—AGE 192 DAYS		
2.3 pounds cottonseed meal daily			2.1 pounds cottonseed meal daily			2.0 pounds cottonseed meal daily		
Time after feeding dye		Color	Time after feeding dye		Color	Time after feeding dye		Color
hours	minutes		hours	minutes		hours	minutes	
6	45	0	2	30	0	2	30	0
10	15	0	7	30	0	7	30	0
11	45	0	9	25	0	10	50	0
15	0	+++	10	30	0	12	0	+
17	30	++++	12	0	0	14	0	++
18	0	++++	16	0	++++	16	30	++++
23	0	++++	19	10	++++	19	0	++++
23	45	++++	23	0	++++	22	30	++++
25	0	++++	34	20	++++	23	0	++++
33	0	++++	37	0	+++	25	15	++++
34	30	++++	40	10	++	27	0	++++
36	0	++++	42	35	+	33	30	++++
41	0	+++	47	0	+	37	0	++++
47	0	++	56	30	0	40	0	++
48	25	++				42	40	+
51	0	+				47	0	+
56	0	0				48	0	0
58	30	0				50	45	0
						56	30	0

TABLE 5—*Continued*

Lot 2

G 2—AGE 256 DAYS			G 4—AGE 226 DAYS			G 6—AGE 117 DAYS		
2.9 pounds linseed oil meal daily			2.7 pounds linseed oil meal daily			0.6 pound linseed oil meal daily		
Time after feeding dye		Color	Time after feeding dye		Color	Time after feeding dye		Color
hours	*minutes*		*hours*	*minutes*		*hours*	*minutes*	
7	30	0	2	30	0	2	30	0
10	15	0	7	30	0	7	30	0
11	45	0	10	30	0	9	0	0
15	0	++	13	45	+++	10	30	0
17	30	+++	17	25	++++	12	0	0
23	0	++++	19	0	++	14	5	+
25	0	++++	22	30	++++	15	0	++
28	30	++++	23	0	++++	16	30	++
33	0	++++	25	15	++++	18	40	+++
41	0	++++	30	0	++++	23	0	++++
47	0	++	33	30	+++	25	15	++++
50	30	+	36	0	+++	27	0	++++
56	0	0	40	10	+++	34	10	++++
57	45	0	42	0	++	37	0	++++
58	30	0	46	30	+	37	10	++++
62	30	0	47	0	+	40	15	++++
			54	25	0	42	45	++++
			56	30	0	48	0	++++
			60	10	0	56	55	++
						60	15	+

++++ abundant dye; +++ large amount; ++ relatively large; + small amount; 0 absent.

fed. Ten recognized judges of cattle attempted to differentiate between the heifers fed linseed and those fed cottonseed meal. The difference between the sleekness of coat, pliability of hide, and condition of the animals was so small that the judges were unable to distinguish between the two lots of heifers. The double thickness of hide was measured from time to time by a pair of calipers. The measurement was taken at the mid-section of the last rib. No difference in double thickness was observed between the animals fed linseed and those fed cottonseed meal.

Lice infestation in cattle depends to some extent on the health of the skin. When the sebaceous glands secrete sufficient oil to

keep the hair oily, lice can not exist. The oil clogs up the breathing mechanism of the lice, therefore, any feeding regime which keeps down lice infestation probably does so by increasing the secretory activity of the sebaceous glands of the skin. In this experiment, there was no appreciable difference in lice infestation between the linseed and the cottonseed meal fed heifers.

The rate of food passage through the digestive track. It is common opinion among investigators that linseed oil meal has a laxative effect and cottonseed meal a costive effect when fed to cattle. Therefore, in this work the rate of food passage through the digestive tract and the consistency of feces of the two lots of heifers were studied.

Several methods have been suggested and tried for determining the rate of food passage in ruminants. None of these has proved satisfactory.

Ewing and Smith (38) attempted to use small rubber washers as markers. Some of the rubber washers were passed in from twelve to sixty hours, and some were not passed at all. These investigators later adopted the slaughter method. Fish (39) fed Sudan III to animals prepared for slaughter and noted the presence of the dye in various portions of the digestive tract.

A modification of the method suggested by Fish was used in this work. One gram of the fat soluble dye Sudan III was shaken with 100 cc. of ethyl ether. This amount of ether did not dissolve all the dye. However, the dye was held in suspension for a short time. This dye suspension was quickly and thoroughly mixed with a feed of either linseed or cottonseed meal. The ether was permitted to evaporate from the feed. The feed containing Sudan III was fed in the morning immediately following the feeding of silage.

Samples of feces were then collected from each passage and the time noted. These samples were dried in an oven at 80 to 90°C. One gram of the dried feces was then extracted with 10 cc. of ethyl ether. The residue was filtered off. The color of this extract from the first sample of feces was green, due to the cholorphyll from the hay. The red color of Sudan III superimposed upon the green produced a light brown to a brownish red color.

The color of the ether extracts of the feces samples was compared in order to determine the rate of food passage of the two rations. Only three animals in each group were used in the first trial. The results are reported in table 5.

The average initial appearance of the dye in the feces was fourteen hours, twenty minutes in both groups. The average final appearance of the dye was fifty-two hours, thirty-five minutes in the group receiving linseed oil meal and forty-eight hours, twenty minutes in the group receiving cottonseed meal. A similar trial was conducted on all ten heifers eight months later when the animals in lot 1 were receiving 2.4 pounds of cottonseed meal per head and those in lot 2 were receiving 3 pounds in linseed oil meal per head. The initial appearance of the dye in this trial was approximately fifteen hours, thirty minutes and the final appearance was fifty-five to sixty hours in both groups.

Thus far in this work there has been very little difference in the consistency of feces excreted by animals receiving cottonseed meal and linseed oil meal. The feces of all the animals have been normal throughout the experiment.

DISCUSSION

There appears to be a close relationship between the symptoms of cottonseed meal injury in cattle and the injury produced when too much concentrates are fed in proportion to roughage. Convulsions, stiffness, and blindness which are the common symptoms of cottonseed meal injury in cattle are also manifested when the ration contains a considerable amount of concentrates and little or no hay or grass. Blindness and stiffness of gait were manifested by the two calves C 44 and C 45 even though no cottonseed meal was fed. These findings are in harmony with those of the North Carolina Station (36) where similar symptoms were produced in cattle fed either soybean meal or peanut meal with a roughage of poor quality. The cause of the deficiency in cattle fed such rations is not definitely known.

Davenport (40) concluded from his investigations that bulk was necessary. McCandlish (41) observed that calves could not be grown from birth to maturity on milk alone. The addition of

certain grains only intensified the deficiency. Vitamin supplements were of little value. Eckles (42) reported favorable results when calcium carbonate was added to the ration of a calf. However, the addition of vitamins had no apparent beneficial effect. Huffman and Robinson (43) were unable to raise calves on concentrates alone or with concentrates supplemented with calcium carbonate, bone meal, or cod liver oil. The addition of bone meal to the ration of C 70 failed to prevent the symptoms of cottonseed meal poisoning.

Our results suggest that cottonseed meal may be fed to growing calves without injury. The five heifers receiving 2.4 pounds of cottonseed meal throughout most of their growing period along with an otherwise adequate ration failed to show the symptoms of cottonseed meal injury. Although the gain made by this group was not as great as that made by the animals receiving linseed oil meal, the difference was negligible in view of the fact that the smaller and slightly less thrifty calves received cottonseed meal in the ration. Since the animals in this group are all above normal in weight, the difference in the two groups can easily be explained on the basis of individual variations. The purpose of this work was not to bring out the relative efficiency of the proteins of cottonseed meal and linseed meal.

The results of this experiment indicate that at least two pounds of cottonseed meal per day can be fed without injury to calves which have reached five months of age, when plenty of good hay is fed along with silage. Apparently, roughage in sufficient quantity and of the proper quality neutralizes the bad effects of concentrates.

These results are in harmony with the results obtained by the North Carolina Experiment Station (36) where such supplements as pasture, hay, minerals, and vitamins made possible the heavy feeding of cottonseed meal without injurious results. Gossypol, which many investigators have found to be the poisonous principle in the seed of cotton, is probably not the cause of cottonseed meal injury brought about by the heavy feeding of cottonseed meal to cattle. So-called cottonseed meal injury in cattle is probably due to dietary deficiencies in the ration. The results

of this experiment indicate that such deficiencies in cattle may be overcome by feeding a sufficient amount of corn silage and hay of good quality.

The results of this investigation are not in harmony with the generally accepted idea that the feeding of linseed oil meal makes a sleeker coat and a more pliable hide than when cottonseed meal is fed. There was very little difference between the two lots in this respect. These results agree with the findings of Curtiss (45) who concluded that cottonseed meal had the same effect when fed to horses as linseed oil meal, making the coat smooth and glossy.

The results also failed to demonstrate the idea that linseed oil meal has a more laxative effect than cottonseed meal. There was no appreciable difference between the two groups in the rate of food passage, either in the initial appearance of a feed in the feces or in the lag of feed in the digestive tract. About fifteen hours were required in both groups of animals for the initial appearance of the dye used as an indicator and the lag was from fifty to sixty hours in all cases. The consistency of the feces in the two groups was practically the same even when the lot which was fed cottonseed meal received 2.4 pounds of cottonseed meal daily and the other group received three pounds of linseed meal daily. However, animal C 43 had diarrhea from time to time on a ration consisting of 6 pounds of cottonseed meal daily along with corn, oats, and wheat straw. Other investigators have observed this condition among cattle fed heavily on cottonseed meal. (32) (44) Curtis (45) reported that cottonseed meal was laxative when fed to horses.

SUMMARY

Cottonseed meal injury in cattle is similar, if not identical, to the injury produced when too much concentrates in proportion to roughage is fed.

At least two pounds of cottonseed meal daily can be fed to calves five months of age or older which receive all the silage and hay of good quality they will eat.

There was no appreciable difference in the sleekness of coat

and pliability of hide between the heifers receiving cottonseed meal and linseed meal.

There was no appreciable difference between the rate of food passage through the digestive tract by the heifers receiving cottonseed meal and linseed oil meal.

There was no measurable difference in the consistency of feces exceted by the two groups of heifers.

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PLATE 1

CROSS-SECTION OF OPTIC NERVE OF ANIMAL C 43 ENLARGED 125 TIMES, SHOWING DEGENERATION OF MYELIN SHEATHS IN CENTER OF OPTIC NERVE

This animal received a ration of cottonseed meal, corn, oats, and wheat straw. G 1, G 3, G 5, G 7, and G 9 received cottonseed meal for nine months after the experiment started from the same shipment fed C 43.

PLATE 2

SHOWING CONSTRICTION OF OPTIC NERVE IN ANIMAL C 45, ENLARGED 30 TIMES

This animal received a ration of corn gluten, corn distillers grain, corn, oats, and wheat straw.

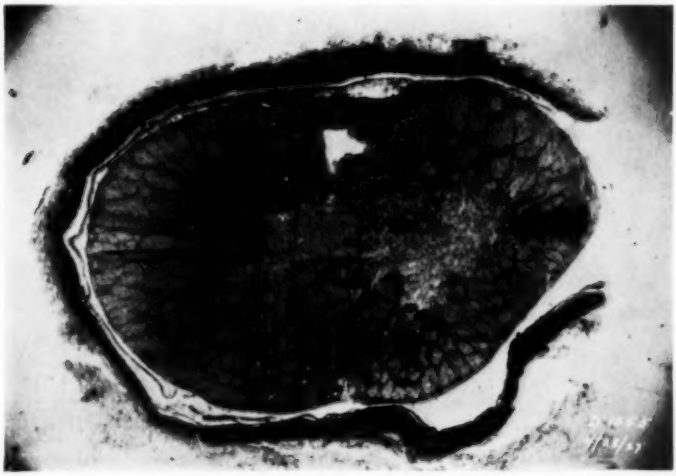

PLATE 1

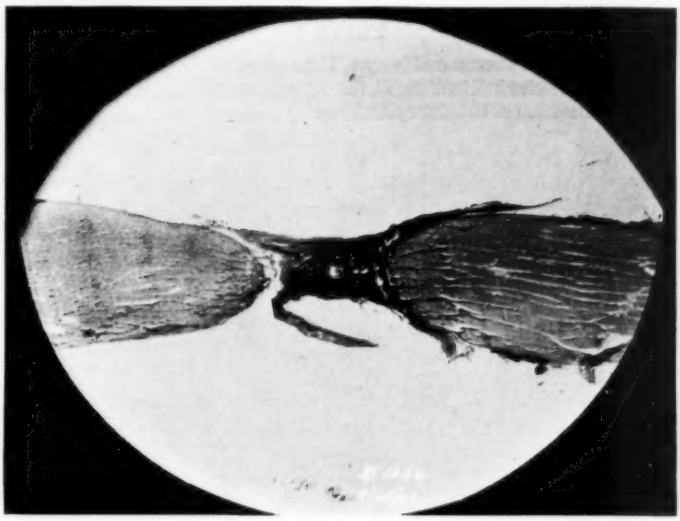

PLATE 2

PLATE 3

C 43. Section of Kidney, Enlarged 800 Diameters

A, intertubular proliferation; *B*, thickened and distorted Bowman capsule; *C*, parenchymatous degeneration of tubular epithelium.

PLATE 4

C 45. Section of Kidney, Enlarged 800 Diameters

A, focus of cellular infiltration; *B*, thickened Bowman's capsule; *C*, parenchymatous degeneration of tubular epithelium.

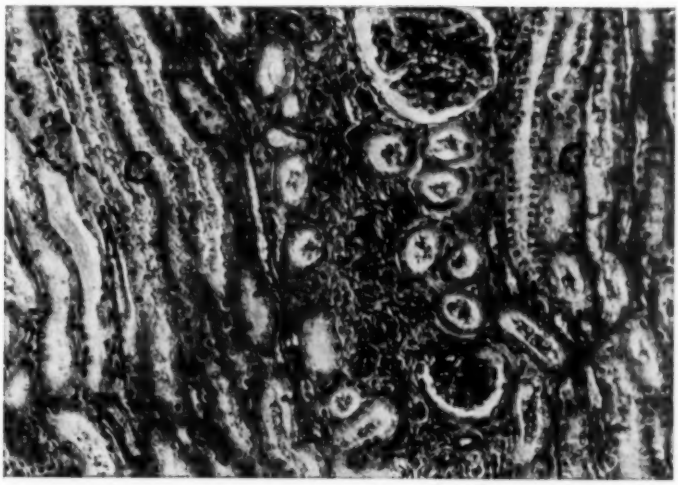

PLATE 3

PLATE 4

PLATE 5

C 43. Section of Kidney, Enlarged 1000 Diameters

Focus of cellular infiltration

PLATE 6

C 45. Section of Kidney, Enlarged 750 Diameters

Focus of cellular infiltration

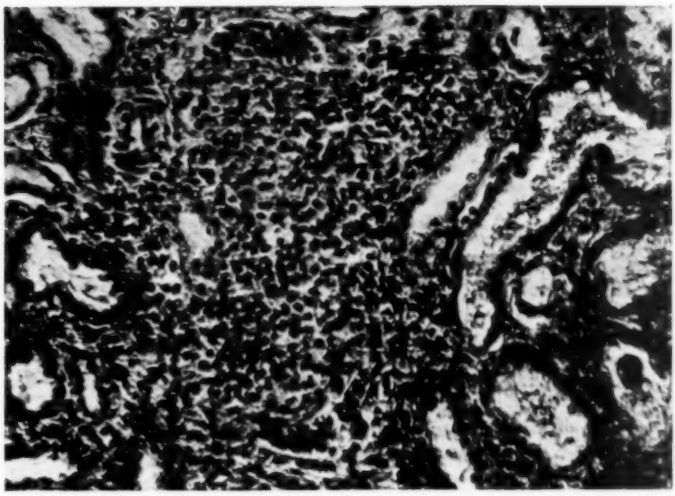

PLATE 5

PLATE 6

PLATE 7

G 3 AT BREEDING AGE (FIFTEEN MONTHS)
Principal source of protein: Cottonseed meal

PLATE 8

G 4 AT BREEDING AGE (FIFTEEN MONTHS)
Principal source of protein: Linseed meal

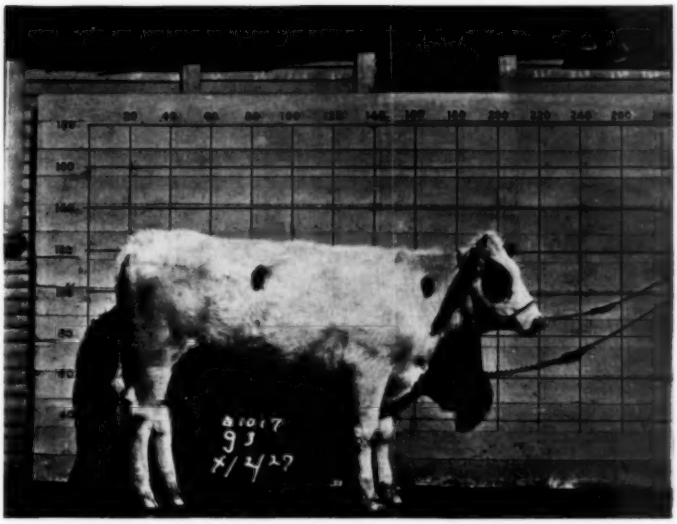

PLATE 7

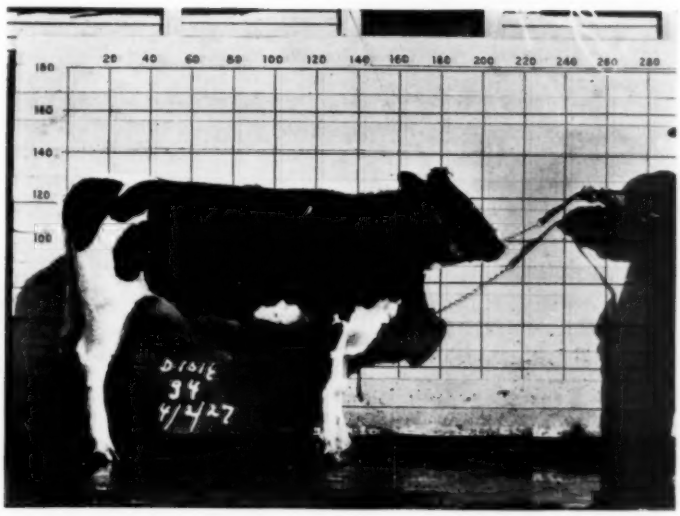

PLATE 8

SOME OBSERVATIONS ON THE BACTERIAL CONTENT OF DRIED MILK*

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The removal of the water from milk during the process of drying leads to transportation economies, and enhanced keeping qualities. The commercial life of fluid milk is decidedly limited, particularly because many microörganisms are capable of rapid alteration of a food stuff with such a high water content. The development of microörganisms in the dehydrated product is precluded since the percentage of moisture in the powdered milk is far below the requirements of the organisms commonly present in milk. Consequently, it is to be expected that deterioration of dried milk must be explained on bases other than microbiological.

However, the bacterial content of the dried milk should be taken into account, especially the survival of microörganisms during long periods of storage and the possibility of subsequent fermentation caused by them when the product is reconstituted by the addition of water up to the normal concentration of moisture of fluid milk.

With this point in mind a number of samples of dried milk purchased in local stores or obtained directly from the manufacturers or distributors have been studied to determine the bacterial content, before and after periods of storage.

REVIEW OF LITERATURE

A considerable volume of literature has accumulated upon the manufacture, composition and utilization of dried milk. Data upon the bacterial content of this product, however, are not very extensive.

Delepine (3) has reported upon some careful studies of the effect of different methods of manufacture upon the bacterial content of

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milk during the drying process, with particular emphasis placed upon the destruction of the tubercle bacillus. He has shown that a whole milk dried by the spray process yielded a product which contained from 10,000 to 15,200 bacteria per gram when taken directly from the drying chamber. Downs (4) found that the bacterial count of spray process powder, prepared from milk previously pasteurized and condensed, ranged from 15,600 to 595,000 per gram, with an average of 178,000. Milk dried by the same process, but not condensed before subjection to the spraying process yielded a product containing 1,500,000 to 3,500,000 bacteria per gram or an average of 2,269,000. Tillmans and Strohecker (12) report counts of 5000 to 30,000 per gram for powder prepared by the Krause process, where the milk was delivered into a drying chamber from rapidly rotating discs.

An anonymous report (1) from England on milk dried by the Hatmaker or hot drum process indicates that the product so obtained gave a count of 70 to 120 per cubic centimeter when reconstituted to the normal moisture content of fluid milk. Delepine (3) found that dried whole milk directly from the rollers (Just-Hatmaker process), contained 70 to 300 bacteria per gram but after it had been powdered the count had increased to 5950 to 14,600. Dried skim milk prepared in the same way gave a count of 10 to 100 per gram when taken from the rollers. Downs (4) studied powder made by the modified Kunick process and found 16,900 to 626,000 bacteria per gram with an average of 49,500. The experiments of Grosso (5) upon Just-Hatmaker dried milk revealed 4000 to 5400 per gram. Hoffman (6) made counts upon the product made by the same process and found 4000 to 5000 per gram in dried whole milk and 2200 to 6000 per gram in dried skim milk. One sample of Just-Hatmaker powder studied by Hueppe (7) gave maximum counts of 4100 per gram on gelatin plates, and 6400 on agar. The minimum counts were 800 to 1000 per gram. Jephcott and his co-workers (8) report the counts obtained from reconstituted milk made from dried milk manufactured by the drum process during three autumn months. The counts in September were 0 to 565 per cubic centimeter, in October, 0 to 275 and in November, 0 to 198, with an average for the three months of 25 per cubic centimeter. Kossowicz (9)

found that Just-Hatmaker dried milk contained 45 to 80 bacteria per gram when the product was taken directly from the drums and 750 to 1,250 per gram when taken from the collecting boxes. Supplee and Ashbaugh (11) made analyses upon a considerable number of samples prepared by the Just-Hatmaker process. The counts obtained from 158 samples, within one day after manufacture varied from 350 to 170,000 per gram, averaging 10,100. Ten samples taken directly from the cylinders averaged 561 per gram. Seven samples were also studied to show progressive contamination in handling. The dried milk taken directly from the drums averaged 563 per gram, after sifting 1614 and after packing 3271 bacteria per gram.

A report by Coutts (2) gives the counts obtained from a number of samples of dried milk but the process by which they were made is not mentioned. Full cream dried milk reconstituted (1:9) gave counts of 0 to 11,900 per cubic centimeter for aerobes at 37°C. for forty-eight hours incubation; 100 to 86,400 per cubic centimeter at 22°C. for ninety-six hours; for anaerobes 0 to 300 per cubic centimeter at 37°C. for forty-eight hours; and 0 to 890 per cubic centimeter at 22°C. for ninety-six hours. Five samples of the completely or partially skimmed product when reconstituted (1:7) showed 0 to 892,000 bacteria per cubic centimeter for aerobes at 37°C. for forty-eight hours incubation; and 100 to 757,200 per cubic centimeter at 22°C. for ninety-six hours; for anaerobes 0 to 38,800 per cubic centimeter at 37°C. for forty-eight hours and 0 to 59,960 per cubic centimeter at 22°C. for ninety-six hours. Likewise, Prachfeld (10) reports for commercial powder, without stating the process by which it was made, 4071 to 21,700 bacteria per gram of powder.

Delepine (3) observed the changes taking place in the bacterial count of drum-dried whole milk during a storage period of 112 days. The counts are based upon the number of bacteria per cubic centimeter of reconstituted milk and are as follows:

	COUNT PER CUBIC CENTIMETER	
	Gelatin plates 20°C.	Agar plates 37°C.
Fresh	4,900	800
112 days	4,180	500–600

Kossowicz (9) found that dried milk which contained 750 to 1,250 bacteria per gram before storage, showed after two months a count of 450 to 930 per gram, while that preserved in soldered tin boxes had a count of 1,850 to 2,460 per gram. Supplee and Ashbaugh (11) studying 43 samples of Just-Hatmaker dried milk during a period of a year obtained the following average counts per gram of powder:

FRESH	2 MONTHS	4 MONTHS	6 MONTHS	8 MONTHS	10 MONTHS	12 MONTHS
22,508	7,818	2,487	655	412	354	261

They point out that there is some difference in the rapidity of the decrease in count depending upon the moisture content of the sample.

EXPERIMENTAL DATA

Sources of samples

For the studies which are reported herewith, samples of dried milk were obtained from a variety of sources. Among them were several samples from Canada, Holland, Italy and New Zealand. The majority of the samples were purchased locally, some were presented by the manufacturers, while others had been on the shelves of the laboratory for several years. Few of the samples gave any clue as to the date of manufacture, consequently nothing was known about their past history. They were simply representative samples that might be found on the shelves of any market.

Methods of analysis

The containers were thoroughly wiped with a 5 per cent phenol solution, wiped dry and flamed carefully before they were opened. Wherever a can opener was required, it was sterilized before use. After the containers were opened, they were protected from contamination while being mixed thoroughly with a sterile wooden spatula. A 10-gram sample was weighed on sterile oiled weighing paper then placed in a 250 cc. wide-mouth, glass stoppered bottle. Ninety cubic centimeters of sterile water warmed to 35 to 40°C.

were added slowly. The dried milk was dissolved as thoroughly as possible and platings made with the necessary dilutions. Clay-top plates were used in order to reduce spreaders to a minimum. The plates were poured with milk powder agar and

TABLE 1

Bacterial content of domestic dried milk (spray process)

Number of bacterial colonies per gram of dried milk

ORIGINAL	AFTER STORAGE AT ROOM TEMPERATURE FOR						
	6 months	1 year	2 years	3 years	4 years	5 years	6 years
5,500,000 (S)	2,400,000		74,000				1,700
3,100,000 (W)		2,200				1,310	
2,800,000 (W)		36,500	5,000	2,300			
500,000 (W)		226,000					290
490,000 (S)			625,000	164,000	38,000		
400,000 (W)		200				480	
250,000 (S)		400		950		280	
157,000 (S)		162,000	11,100	18,500			
84,000 (W)			4,500	2,800			
54,000 (W)		3,800		2,200			
52,000 (W)			1,590		8,000		
51,000 (W)	3,500	2,800			2,250		
50,000 (W)			650	625	180		
42,000 (W)	19,500		300		100		20
34,000 (W)	15,200	1,500		2,000			
29,000 (W)		3,700	3,000	620			
27,600 (W)	3,100	2,000					
27,600 (W)	5,000			1,850	495		
18,400 (W)	3,000	2,300				170	
8,200 (W)		16,000	60	200			
8,000 (W)	2,700		100		85		10
7,100 (S)		770	165				
6,200 (S)		750	220	180			
4,400 (W)							

W = whole milk; S = skimmed milk.

incubated in 20°C. for five days and for an additional two days at 37°C. after which they were counted. The results are reported in terms of the number of bacterial colonies per gram of powder.

The counts were not always easy to make because of the persistence of spreaders, even when the clay-top plates were used. The dehydration of the agar in the plates was checked as far as

possible by placing open dishes of water on top of each pile of plates. Even with this precaution some plates dried badly. The counts that are reported were made only from satisfactory plates. The results of many analyses had to be discarded because of the drying or the spreaders.

TABLE 2

Bacterial content of foreign dried milk (spray process)

Number of bacterial colonies per gram of dried milk

ORIGINAL	AFTER STORAGE AT ROOM TEMPERATURE FOR			
	1 year	2 years	3 years	4 years
3,100,000 (S)		80,000	350	300
750,000 (W)		20,000	400	120
660,000 (W)		7,700	9,750	
79,000 (W)		1,600	7,000	
61,000 (W)	63,000	6,300	140	
11,000 (S)		1,200	11,800	
5,400 (S)	5,000	2,600	2,100	

W = whole milk; S = skimmed milk.

TABLE 3

Bacterial content of domestic dried milk (drum process)

Number of bacterial colonies per gram of dried milk

ORIGINAL	AFTER STORAGE AT ROOM TEMPERATURE FOR				
	6 months	1 year	2 years	3 years	4 years
2,200 (W)	200	100		80	
2,050 (W)	1,470		200		85
320 (W)			80	60	30
240 (W)			100	90	28
40 (W)		40			

W = whole milk; S = skimmed milk.

Bacterial counts of powders made by the spray process

The original counts on the spray powders show a wide range as indicated in column one of tables 1 and 2. Both the domestic and foreign samples show this tendency. On the plates prepared from the high count samples there was a noticeable predominance of small subsurface colonies, resembling those of *S. lactis*. Sterile

milk inoculated with these colonies, in the majority of cases, was coagulated and acid in reaction. In many of the plates, the flora resembled closely the flora of freshly drawn milk. Samples known to be old had a more restricted flora. It was interesting to find one sample in a sealed can, known to be at least twelve years old, which gave a count of 52,000. The data indicate that the bacterial content of spray powder may be expected to be in

TABLE 4

Bacterial content of foreign dried milk (drum process)

Number of bacterial colonies per gram of dried milk

ORIGINAL	AFTER STORAGE AT ROOM TEMPERATURE FOR			
	1 year	2 years	3 years	4 years
7,900 (W)	140		20	40
7,800 (W)	60		10	70
420 (W)	230	110		60
350 (W)		50	80	10
310 (W)	410	180		50
250 (S)		40	50	25
250 (W)				
240 (W)	170	90		

W = whole milk; S = skimmed milk.

the thousands per gram and sometimes in the millions. This is in accordance with the reports of others. A diminution in the number of bacteria appears to take place more rapidly after the sealed package has been opened one or more times.

Bacterial counts of dried milk made by the drum process

Only a few samples of the drum process product were studied. The results are given in tables 3 and 4. The domestic and foreign samples were quite similar. Most of the original counts were in the hundreds with an occasional sample showing counts of a few thousands. This likewise, corresponds with other data in the literature.

Bacterial counts of dried milk after storage

Many of the samples were retained for a period of time depending upon the quantity of the sample. They were left in a pro-

tected place at room temperature during this time. The samples were kept in the original containers or placed in glass stoppered bottles. Analyses were made at intervals over a period of six years. It was impossible to make a count on every sample each year because the quantity of the samples was limited. Irregular periods of storage for the various samples were decided upon to give a wider range of data.

The results of these analyses are shown in tables 1 to 4. It will be noted that there is a decided decrease in most spray process

TABLE 5

Reduction in counts after various periods of storage

TYPE OF DRIED MILK	AFTER STORAGE AT ROOM TEMPERATURE FOR						
	6 months	1 year	2 years	3 years	4 years	5 years	6 years
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Spray	56.3	90.7	93.9	97.4	99.2	99.9	99.9
Drum	60.0	96.0	97.6	97.9	97.9		

TABLE 6

Types of bacteria present in dried milk

TYPE OF DRIED MILK	NEUTRAL	NEUTRAL PEPTONIZING	WEAK ACID	ACID COAGULATING	ACID PEPTONIZING	ALKALINE	ALKALINE PEPTONIZING
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Spray	11.0	2.4	26.0	26.8	12.8	7.3	13.7
Drum	22.5	6.2	15.1	5.2	17.4	8.9	24.7

samples during the first year of storage. This continues to be the case after increasingly long periods of storage.

In the dried milk from the drum process the decrease is not as great, relatively, as in the spray process powders. There is apparently a greater variety of organisms that have resisted drying originally in the spray process. Those which remain in the drum process product after the exposure to the high temperatures of the drum are probably principally spore-forming types which are able to withstand the conditions during storage. Unreported studies of the organisms present in dried milks after

several years of storage reveal largely Gram-positive, spore-forming rods, although some coccus-forms persist.

Table 5 presents the percentage reduction in count in the various groups of dried milks during the six-year period. The data point out clearly the high death rate during the first year.

Types of bacteria present in dried milk

A study was also made upon the percentage distribution of various types of organisms in the different groups of dried milk at the time the original analysis was made. The figures represent

TABLE 7

Effect of storage temperatures upon the bacterial content of dried milk

TYPE OF DRIED MILK	TEMPERATURE OF STORAGE	NUMBER OF BACTERIAL COLONIES PER GRAM		REDUCTION
		Original	After 1 year	
	°C.			*per cent*
Spray (atomized)	5	33,500	4,100	87.7
	10		1,500	95.2
	20		1,200	96.4
	37		900	97.3
Spray (centrifugal)	5	27,600	3,700	86.6
	10		2,000	92.7
	20		1,500	94.5
	37		1,000	96.4
Drum	5	2,200	50	96.8
	10		100	95.4
	20		100	95.4
	37		50	96.8

the averages for all samples. The differentiation of types is based upon the action of the organisms upon litmus milk incubated at 20°C. for five days plus two days at 37°C. All the colonies upon the plates showing counts of 20 to 200 were picked for each sample unless the spreaders made it impossible to do so. In this case a representative plate, not showing spreaders, was selected.

The data given in table 6 indicate a state of affairs which might be expected from a knowledge of the different processes of manu-

facture. The drum process dried milk shows a predominance of peptonizing types, the spray process, of acid producing types.

It may be added that the reconstituted samples were allowed to stand in a refrigerator for two weeks after they were prepared. The majority of these showed an acid coagulation followed by peptonization, and sometimes extensive gas formation. A few of the samples remained neutral or became alkaline in reaction. In every case peptonization took place.

Effect of storage temperatures upon the bacterial content of dried milk

Three samples of dried milk, representing respectively the atomized spray, centrifugal spray, and drum process were divided into four lots, and stored in sealed porcelain jars for one year at 4°C., 10°C., 20°C., and 37°C. respectively.

The results are given in table 7. It will be noted that in general the samples stored at 37°C. showed the greatest reduction in count while those kept at 5°C. showed the least reduction. This is especially true of the spray process powders. There is not as much difference in the drum process samples probably because the resistant types of bacteria are the ones to survive originally the drying process.

SUMMARY

1. The bacterial counts of 31 samples of dried milk prepared by the spray process, and 13 samples prepared by the drum process are reported, before and after storage periods up to six years.

2. The original counts on the spray process samples ranged from 4400 to 5,500,000 per gram, with the majority showing counts above 50,000.

3. The original counts on the drum process samples ranged from 40 to 7,900 per gram, with the majority showing counts below 500.

4. A remarkable decrease in counts occurred in both types of dried milk after a storage period of one year.

5. The spray process powders showed a more marked reduction in counts after a two-year period of storage than did the drum

process powders. The maximum reduction in counts (99.9 per cent) for the spray process powders took place after five years. In the case of the drum process samples the maximum (97.9 per cent) was reached after three years.

6. There was a significant difference in the types of bacteria present in the dried milk prepared by the two processes.

7. High temperatures seemed to be more effective in bringing about reductions in counts during storage.

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PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The annual meeting of the American Dairy Science Association was held at the University of Wisconsin, Madison, Wisconsin, on June 26, 27 and 28. The program committee consisted of H. A. Ruehe of Illinois, C. H. Eckles of Minnesota, and C. C. Hayden of Ohio, who arranged to carry out a program in a similar manner to that presented last year at the summer meeting in Michigan.

The meeting began at 9:15 on June 26 with Professor H. C. Jackson of Wisconsin presiding instead of President G. C. White of Connecticut who was unable to attend. It was fitting that Professor Jackson should have presided at this meeting and that Dean H. L. Russell should have presented the opening remarks since the first dairy school in America was held at the University of Wisconsin for the purpose of giving instruction in the Babcock test. The building in which the meeting was held was an annex to the first dairy building constructed in this country.

During the morning's session communications were presented from President G. C. White, Vice-President A. C. Baer, Secretary-Treasurer J. M. Sherman, and the chairman of the program committee H. A. Ruehe, advising the Association of their inability to attend the meeting and expressing their hope that the program would prove interesting and profitable. The meeting was unusual on account of the absence of the executive committee.

At noon the members of the Association were given a luncheon at the experimental farms of the Quaker Oats Company. Before returning to the University, the experiments in feeding oat hulls to dairy cattle and other farm live stock were explained to the visitors.

The afternoon's session was devoted principally to nutrition studies. E. L. Anthony of West Virginia presided at the meeting. At the conclusion of this meeting the members of the Association

were taken to the experimental barns of the University of Wisconsin in which a new experiment on nutrition and contagious abortion is being conducted. About 50 Holstein heifers were raised on a poor and a good dairy ration, according to E. B. Hart of the Division of Agricultural Chemistry, and these heifers, now in their first period of lactation, are being fed on these widely different rations Within another year after the heifer production records are available, the cattle will be infected with contagious abortion for the purpose of determining the effect of nutrition upon the degree of infection from this disease.

On the evening of the same day, a banquet was planned at the Park Hotel for 6:30. A brief entertainment was given during the course of the dinner followed by several very short addresses. The real feature of the banquet was the presence of Dr. S. M. Babcock, emeritus professor of the University and originator of the test for fat in milk and cream which bears his name.

The banquet was adjourned early in the evening to permit the holding of committee meetings. The executive committee with associate editors and officers of the various sections met for the purpose of transacting necessary business. The meeting was attended by Ragsdale, Price, Anthony, Williams, Hastings, Lucas, Baltzer, and Dahlberg. Dahlberg acted as chairman and Price as secretary. The committee considered certain matters concerning publication of the JOURNAL OF DAIRY SCIENCE and the selection of committees on nominations and resolutions.

The general session held on the morning of June 27 was the last general meeting prior to the division of the membership into its various sections. In the absence of A. C. Baer of Oklahoma, A. C. Dahlberg of Geneva presided. The following committees were named.

NOMINATING

E. S. Guthrie, chairman
P. S. Lucas
A. C. Ragsdale

RESOLUTIONS

E. L. Anthony, chairman
A. C. Ragsdale
A. C. Baltzer
W. V. Price

Resolutions were presented at the close of this session and the nominating committee gave the following report.

President.....{G. C. White of Connecticut / J. R. Dice of North Dakota

Vice-President.....{H. W. Gregory of Indiana / H. A. Ruehe of Illinois

The offices of secretary-treasurer and editor being held for two years, were not vacant at this time.

The dairy manufacturers section held its meeting on the afternoon of June 27 with W. V. Price of New York presiding and its meeting on the morning of June 28 with H. C. Jackson of Wisconsin presiding. One of the interesting features of this program was the report of the research committee of the International Association of Ice Cream Manufacturers given by H. F. Judkins of Springfield, Mass. This committee would welcome the coöperation of any investigator in agricultural college work who is interested in a problem of value to the ice cream industry. This committee would be glad to furnish research problems with brief outlines concerning the methods of attack or would be glad to go over existing problems for the purpose of assisting the investigator in arriving at conclusions of value to the industry. The committee would also be willing to read and comment upon manuscripts giving the results of investigational work with the intention of offering suggestions from the standpoint of the industry. Such active coöperation of an industry is especially gratifying to members of the Association and the dairy manufacturers section went on record as approving most heartily this type of coöperation. The following officers were elected for the ensuing year.

Chairman.....W. H. Martin of Kansas
Secretary.....J. C. Hening of New York

The sections of Production, Extension, and Official Testing held joint sessions with G. A. Williams presiding on Wednesday and A. C. Ragsdale on Thursday. The last two sections men-

tioned voted to continue their present officers while the Production section elected the following officers:

Chairman.....Fordyce Ely of Kentucky
Secretary.....R. B. Becker of Oklahoma

The following program was presented:

GENERAL SESSION

H. C. Jackson, presiding

Opening remarks—Responsibilities of dairy staffs
Dean H. L. Russell, Univ. of Wisc.
President's address.....G. C. White, Conn. Agr. College
Announcements
Coming educational problems in dairying
Director K. L. Hatch, Univ. of Wisc.
The curricula on dairying.....H. A. Bendixen, Wash. State College
Teaching dairy cattle judging.....Fordyce Ely, Univ. of Ky.
Correspondence courses in dairy husbandry.....J. R. Dice, N. D. Agr. College
Methods of teaching dairy cattle management.....H. P. Davis, Univ. of Nebr.

E. L. Anthony, presiding

An economic research program for the dairy industry
F. A. Buechel, U. S. D. A., Bureau of Agr. Economics
The relative anti-neuritic and anti-pellagra potency of cow's milk
W. E. Krauss and C. H. Hunt, Ohio Exp. Station
Effects of feeding vitamin A deficient rations to dairy heifers
S. I. Bechel, Pa. Exp. Station
Vitamin E experiments with different feeds.....H. P. Davis, Univ. of Nebr.
A chemical study of fermentation as applied to dairy feeds
A. E. Perkins, Ohio Exp. Station
Nutrients required for normal growth in dairy heifers
T. W. Gullickson, Univ. of Minn.

A. C. Dahlberg, presiding

Calcium assimilation as indicated by bone analysis in long time experiments
E. B. Meigs and A. Hartman, U. S. D. A.
A comparison of cane and kafir silage in dairy rations
R. B. Becker, Okla. A. and M. College
From what age can we raise calves on a dry grain ration
C. B. Bender, N. J. Agr. College
Management as a factor in abortion elimination from dairy herds
H. O. Henderson and E. L. Anthony, W. Va. College of Agr.
The effect of heavy concentrate feeding on the health of dairy cattle
C. F. Huffman, Mich. Agr. College
Value of rice by-products for dairy cows.....H. E. Dvorachek, Univ. of Ark.
Grinding of roughage for dairy cows.....T. M. Olson, S. D. Agr. College

Use of soybean hay and soybean meal in the dairy ration and their effect upon the quality of dairy products produced
W. B. Nevens and P. H. Tracy, Univ. of Ill.
Fetal development of the mammary gland in cattle
C. W. Turner, Univ. of Mo.
The mammary gland and milk secretion.....W. W. Swett, U. S. D. A.
The character of the material on the surface of the fat particle of milk
W. E. Petersen, Univ. of Minn.

DAIRY MANUFACTURERS SECTION

W. V. Price, presiding

Activities of the Research Committee of the International Association of Ice Cream Manufacturers
H. F. Judkins, General Ice Cream Corp., Springfield, Mass.
The relation of preheating and homogenization to the heat stability of cream
C. E. Holm, B. H. Webb, and E. F. Deysher, U. S. D. A.
Cream feathering.....P. H. Tracey, Univ. of Ill.
Does pasteurization make the mineral content of milk less assimilable
M. J. Prucha, Univ. of Ill.
The application of steam for heating and sterilizing dairy equipment
A. W. Farrell, Univ. of Calif.
The effect of hydrogen ion concentration on time of churning cream
E. S. Guthrie and P. F. Sharp, Cornell Univ.
Discussion on the judging of ice cream.....H. P. Judkins

H. C. Jackson, presiding

The effect of different homogenizing pressures on ice cream mix and resulting ice cream, when the percentage of butterfat is varied
W. H. E. Reid, Univ. of Mo.
The use of dry skim milk in ice cream
W. V. Price and R. Whitaker, Cornell Univ.
The effect of certain salts on the properties of ice cream mixes
J. C. Hening, N. Y. Agr. Exp. Station
The grading of commercial gelatine and its use in the manufacture of ice cream
A. C. Dahlberg, N. Y. Agr. Exp. Station
The freezing out of flavors in ice cream.....P. S. Lucas, Mich. Agr. College
A suggested rapid method for determining fat in ice cream
O. F. Garrett, Univ. of Ill.
Business session of dairy manufacturer's section

EXTENSION, PRODUCTION, AND OFFICIAL TESTING SECTIONS

G. A. Williams, presiding

State and county scrub bull eradication campaign
L. A. Higgins, Miss. A. and M. College
Discussion led by Ed. Prewitt, College of Agr., Lexington, Ky.
Missouri better bull program.....M. J. Regan, Univ. of Mo.
Organization and operation of bull associations
S. J. Brownell, State College, Pa.

Developing a state program for proving and retaining dairy sires
E. T. Wallace, Purdue Univ.
Discussion led by George Girrbach, Mich. State College
Report of short cut methods in testing.....S. R. Searles, Univ. of Minn.
Tester training and conferences.....A. C. Baltzer, Mich. State College
Discussion led by Burt Oderkirk, Iowa State College

A. C. Ragsdale, presiding

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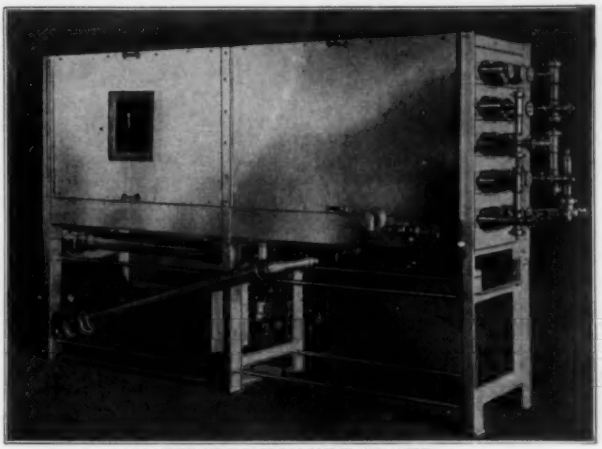

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